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(21) International Application Number: <b>PCT/US95/02203</b>		(72) Inventors: DE PLAEN, Etienne; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). BOON-FALLEUR, Thierry; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). LETHE, Bernard; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). SZIKORA, Jean-Pierre; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). DE SMET, Charles; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). CHOMEZ, Patrick; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). GAUGLER, Beatrice; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). VAN DEN EYNDE, Benoit; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). BRASSEUR, Francis; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). PATARD, Jean-Jacques; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). WEYNANTS, P.; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). MARCHAND, M.; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). VAN DER BRUGGEN, Pierre; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE).	
(22) International Filing Date: 23 February 1995 (23.02.95)		(74) Agent: HANSON, Norman, D.; Felfe & Lynch, 805 Third Avenue, New York, NY 10022 (US).	
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(71) Applicant: LUDWIG INSTITUTE FOR CANCER RESEARCH [US/US]; 1345 Avenue of the Americas, New York, NY 10105 (US).			
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(54) Title: DETERMINATION OF CANCEROUS CONDITIONS BY MAGE GENE EXPRESSION			
(57) Abstract <p>A method for determining cancers is described. The method involves assaying for expression of a gene coding for at least one of MAGE tumor rejection antigen or its precursor expression product.</p>			

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## DETERMINATION OF CANCEROUS CONDITIONS BY MAGE GENE EXPRESSION

FIELD OF THE INVENTION

5        This invention relates to general methods for diagnosing cancers via determining expression of at least one member of the MAGE family of tumor rejection antigen precursors. More particularly, cancers such as lung adenocarcinoma, neck, squamous cell, prostate, and bladder  
10      cancers can be diagnosed by determining expression of one or more members of this family of genes. Also a part of the invention are primers which can be used in these methods, such as amplification methods, of which the polymerase chain reaction ("PCR") is the most well known.

15      BACKGROUND AND PRIOR ART

      The study of the recognition or lack of recognition of cancer cells by a host organism has proceeded in many different directions. Understanding of the field presumes some understanding of both basic immunology and oncology.

20      Early research on mouse tumors revealed that these displayed molecules which led to rejection of tumor cells when transplanted into syngeneic animals. These molecules are "recognized" by T-cells in the recipient animal, and provoke a cytolytic T-cell response with lysis of the transplanted cells. This evidence was first obtained with tumors induced in vitro by chemical carcinogens, such as methylcholanthrene. The antigens expressed by the tumors and which elicited the T-cell response were found to be different for each tumor. See  
25      Prehn, et al., J. Natl. Canc. Inst. 18: 769-778 (1957); Klein et al., Cancer Res. 20: 1561-1572 (1960); Gross, Cancer Res. 3: 326-333 (1943), Basombrio, Cancer Res. 30: 2458-2462 (1970) for general teachings on inducing tumors with chemical carcinogens and differences in cell surface  
30      antigens. This class of antigens has come to be known as  
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5 "tumor specific transplantation antigens" or "TSTAs". Following the observation of the presentation of such antigens when induced by chemical carcinogens, similar results were obtained when tumors were induced in vitro via ultraviolet radiation. See Kripke, J. Natl. Canc. Inst. 53: 333-1336 (1974).

10 While T-cell mediated immune responses were observed for the types of tumor described supra, spontaneous tumors were thought to be generally non-immunogenic. These were therefore believed not to present antigens which provoked a response to the tumor in the tumor carrying subject. See 15 Hewitt, et al., Brit. J. Cancer 33: 241-259 (1976).

15 The family of tum<sup>+</sup> antigen presenting cell lines are immunogenic variants obtained by mutagenesis of mouse tumor cells or cell lines, as described by Boon et al., J. Exp. Med. 152: 1184-1193 (1980), the disclosure of which is incorporated by reference. To elaborate, tum<sup>+</sup> antigens are obtained by mutating tumor cells which do not generate an immune response in syngeneic mice and will form tumors 20 (i.e., "tum<sup>+</sup>" cells). When these tum<sup>+</sup> cells are mutagenized, they are rejected by syngeneic mice, and fail to form tumors (thus "tum<sup>-</sup>"). See Boon et al., Proc. Natl. Acad. Sci. USA 74: 272 (1977), the disclosure of which is incorporated by reference. Many tumor types have been 25 shown to exhibit this phenomenon. See, e.g., Frost et al., Cancer Res. 43: 125 (1983).

30 It appears that tum<sup>-</sup> variants fail to form progressive tumors because they initiate an immune rejection process. The evidence in favor of this hypothesis includes the ability of "tum<sup>-</sup>" variants of tumors, i.e., those which do not normally form tumors, to do so in mice with immune systems suppressed by sublethal irradiation, Van Pel et al., Proc. Natl. Acad. Sci. USA 76: 5282-5285 (1979); and the observation that intraperitoneally injected tum<sup>-</sup> cells 35 of mastocytoma P815 multiply exponentially for 12-15 days, and then are eliminated in only a few days in the midst of

an influx of lymphocytes and macrophages (Uyttenhove et al., J. Exp. Med. 152: 1175-1183 (1980)). Further evidence includes the observation that mice acquire an immune memory which permits them to resist subsequent challenge to the same tumor variant, even when immunosuppressive amounts of radiation are administered with the following challenge of cells (Boon et al., Proc. Natl. Acad. Sci. USA 74: 272-275 (1977); Van Pel et al., supra; Uyttenhove et al., supra).

Later research found that when spontaneous tumors were subjected to mutagenesis, immunogenic variants were produced which did generate a response. Indeed, these variants were able to elicit an immune protective response against the original tumor. See Van Pel et al., J. Exp. Med. 157: 1992-2001 (1983). Thus, it has been shown that it is possible to elicit presentation of a so-called "tumor rejection antigen" in a tumor which is a target for a syngeneic rejection response. Similar results have been obtained when foreign genes have been transfected into spontaneous tumors. See Fearon et al., Cancer Res. 48: 2975-1980 (1988) in this regard.

A class of antigens has been recognized which are presented on the surface of tumor cells and are recognized by cytolytic T cells, leading to lysis. This class of antigens will be referred to as "tumor rejection antigens" or "TRAs" hereafter. TRAs may or may not elicit antibody responses. The extent to which these antigens have been studied, has been via cytolytic T cell characterization studies, in vitro i.e., the study of the identification of the antigen by a particular cytolytic T cell ("CTL" hereafter) subset. The subset proliferates upon recognition of the presented tumor rejection antigen, and the cells presenting the antigen are lysed. Characterization studies have identified CTL clones which specifically lyse cells expressing the antigens. Examples of this work may be found in Levy et al., Adv. Cancer Res. 24: 1-59 (1977); Boon et al., J. Exp. Med. 152: 1184-1193 (1980); Brunner et al., J. Immunol. 124: 1627-1634 (1980);

Maryanski et al., Eur. J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982); Palladino et al., Canc. Res. 47: 5074-5079 (1987). This type of analysis is required for other types of antigens recognized by CTLs, including minor histocompatibility antigens, the male specific H-Y antigens, and the class of antigens referred to as "tum-" antigens, and discussed herein.

A tumor exemplary of the subject matter described supra is known as P815. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988); Szikora et al., EMBO J 9: 1041-1050 (1990), and Sibille et al., J. Exp. Med. 172: 35-45 (1990), the disclosures of which are incorporated by reference. The P815 tumor is a mastocytoma, induced in a DBA/2 mouse with methylcholanthrene and cultured as both an in vitro tumor and a cell line. The P815 line has generated many tum<sup>-</sup> variants following mutagenesis, including variants referred to as P91A (DePlaen, supra), 35B (Szikora, supra), and P198 (Sibille, supra). In contrast to tumor rejection antigens - and this is a key distinction - the tum<sup>-</sup> antigens are only present after the tumor cells are mutagenized. Tumor rejection antigens are present on cells of a given tumor without mutagenesis. Hence, with reference to the literature, a cell line can be tum<sup>+</sup>, such as the line referred to as "P1", and can be provoked to produce tum<sup>-</sup> variants. Since the tum<sup>-</sup> phenotype differs from that of the parent cell line, one expects a difference in the DNA of tum<sup>-</sup> cell lines as compared to their tum<sup>+</sup> parental lines, and this difference can be exploited to locate the gene of interest in tum<sup>-</sup> cells. As a result, it was found that genes of tum<sup>-</sup> variants such as P91A, 35B and P198 differ from their normal alleles by point mutations in the coding regions of the gene. See Szikora and Sibille, supra, and Lurquin et al., Cell 58: 293-303 (1989). This has proved not to be the case with the TRAs of this invention. These

papers also demonstrated that peptides derived from the tumor antigen are presented by the L<sup>d</sup> molecule for recognition by CTLs. P91A is presented by L<sup>d</sup>, P35 by D<sup>d</sup> and P198 by K<sup>d</sup>.

5 PCT application PCT/US92/04354, filed on May 22, 1992 assigned to the same assignee as the subject application, teaches a family of human tumor rejection antigen precursor coding genes, referred to as the MAGE family, and their expression in various tumor types. Lung adenocarcinoma is not among these. Several of these genes are also discussed  
10 in van der Bruggen et al., Science 254: 1643 (1991). It is now clear that the various genes of the MAGE family are expressed in tumor cells, and can serve as markers for the diagnosis of such tumors, as well as for other purposes discussed therein. See also Traversari et al.,  
15 Immunogenetics 35: 145 (1992); van der Bruggen et al., Science 254: 1643 (1991). The mechanism by which a protein is processed and presented on a cell surface has now been fairly well documented. A cursory review of the development of the field may be found in Barinaga, "Getting  
20 Some 'Backbone': How MHC Binds Peptides", Science 257: 880 (1992); also, see Fremont et al., Science 257: 919 (1992); Matsumura et al., Science 257: 927 (1992); Latron et al., Science 257: 964 (1992). These papers generally point to  
25 a requirement that the peptide which binds to an MHC/HLA molecule be nine amino acids long (a "nonapeptide"), and to the importance of the first and ninth residues of the nonapeptide.

Studies on the MAGE family of genes have now revealed  
30 that, in some cases a nonapeptide is presented on the surface of tumor cells, and that the presentation of the nonapeptide requires that the presenting molecule be HLA-A1. Complexes of the MAGE-1 tumor rejection antigen (the "TRA" or nonapeptide) leads to lysis of the cell presenting it by cytolytic T cells ("CTLs"). Additional  
35 research has correlated other nonapeptides derived from MAGE and genes to HLA-A1 and other MHC class I molecules.

Research presented in, e.g., U.S. patent application Serial No. 07/938,334 filed August 31, 1992, showed that, when comparing homologous regions of various MAGE genes to the region of the MAGE-1 gene coding for the relevant nonapeptide, there is a great deal of homology.

The nucleic acid sequences which code for the nonapeptides were also described therein. These nucleic acid molecules were described as also being useful as diagnostic probes for tumor presence.

The application also showed how it had been found that a cellular model could be used, wherein a non-human cell can be transfected with a nucleic acid sequence coding for a human HLA molecule. The resulting transfectant could then be used to test for nonapeptide specificity of the particular HLA molecule, or as the object of a second transfection with a MAGE gene. The co-transfectant could be used to determine whether the particular MAGE based TRA is presented by the particular HLA molecule.

Many of the references referred to supra present data on the expression pattern of various MAGE genes in different types of cell lines and tumor tissues. What is evident from these data is that there is no "unifying principle" which allows one to predict which MAGE gene will be expressed by a particular tumor type. Thus, while on one level one can say that MAGE genes are "markers" for tumors, on the level of specific tumor types, the correlation of marker and tumor type is not predictable, and must be determined empirically.

It has now been found that one can carry out cancer determination assays by assaying for expression of one or more members of the MAGE family of tumor rejection antigen precursors. How this is accomplished is shown in the examples which follow.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B depict detection of transfectants expressing antigen P815A.

5 Figure 2 shows the sensitivity of clones P1.HTR, P0.HTR, genomic transfectant P1A.T2 and cosmid transfectant P1A.TC3.1 to lysis by various CTLs, as determined by chromium release assays.

Figure 3 is a restriction map of cosmid C1A.3.1.

10 Figure 4 shows Northern Blot analysis of expression of gene P1A.

Figure 5 sets forth the structure of gene P1A with its restriction sites.

15 Figure 6 shows the results obtained when cells were transfected with the gene for P1A, either isolated from P815 or normal cells and then tested with CTL lysis.

Figure 7 shows lytic studies using mast cell line L138. 8A.

Figure 8 is a map of subfragments of the 2.4 kb antigen E fragment which also express the antigen.

20 Figure 9 shows homology of sections of exon 3 from genes MAGE 1, 2 and 3.

Figure 10 shows the result of Northern blots for MAGE genes on various tissues.

Figure 11 presents the data of Figure 13 in table form.

25 Figure 12 shows Southern Blot experiments using the various human melanoma cell lines employed in this application.

Figure 13 is a generalized schematic of the expression of MAGE 1, 2 and 3 genes by tumor and normal tissues.

Figure 14 shows results from a chromium release assay using CTL clone 20/38 on various cell lines.

5 Figure 15 presents the result of assays undertaken to determine antigenic specificity of CTL clone 20/38.

Figure 16 shows the results obtained when a TNF release assay was carried out on various transfected cells.

10 Figure 17 shows results secured from qualitative PCR assays for MAGE-1 in lung adenocarcinomas.

Figure 18 presents data pertaining to quantitative measurement of MAGE-1 expression in lung adenocarcinomas.

15 Figure 19 shows reverse transcription/PCR amplification production of mRNA extracted from the bladder tumor of a patient referred to as "HM15". This is shown in all lanes marked "R". In lanes marked "D", amplification products of the genomic DNA from the patient are shown.

20 Figure 20 displays the fraction of tumors expressing genes MAGE-1, 2, 3 and 4 among the superficial and invasive transitional cell carcinomas of the bladder.

#### DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Many different "MAGE" genes have been identified, as will be seen from the sequences which follow the application. The protocols described in the following examples were used to isolate these genes and cDNA sequences.

"MAGE" as used herein refers to a nucleic acid sequence isolated from human cells. The acronym "smage" is used to describe sequences of murine origin.

When "TRAP" or "TRAs" are discussed here in as being specific to a tumor type, this means that the molecule under consideration is associated with that type of tumor, although not necessarily to the exclusion of other tumor types.

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Example 1

In order to identify and isolate the gene coding for antigen P815A, gene transfection was used. This approach requires both a source of the gene, and a recipient cell line. Highly transfectable cell line P1.HTR was the starting material for the recipient, but it could not be used without further treatment, as it presents "antigen A", one of four recognized P815 tumor antigens. See Van Pel et al., Molecular Genetics 11: 467-475 (1985). Thus, screening experiments were carried out to isolate cell lines which did not express the antigen and which nonetheless possessed P1.HTR's desirable qualities.

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To do this, P1.HTR was screened with CTLs which were specific for each of tumor antigens A, B, C and D. Such CTLs are described by Uyttenhove et al., J. Exp. Med. 157: 1040-1052 (1983).

To carry out the selection,  $10^6$  cells of P1.HTR were mixed with  $2-4 \times 10^6$  cells of the CTL clone in a round bottom tube in 2 ml of medium, and centrifuged for three minutes at 150xg. After four hours at 37°C, the cells were washed and resuspended in 10 ml of medium, following Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982). Additional information on the CTL assay and screening protocol, in general may be found in Boon et al., J. Exp. Med. 152: 1184-1193 (1980), and Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982), the disclosures of which are incorporated by reference.

When these selections were carried out, a cell line variant was found which expressed neither antigen A or B. Additional selections with CTLs specific for antigen C then yielded a variant which also lacked antigen C. Please see

figure 2 for a summary of the results of these screenings. The variant PO.HTR is negative for antigens A, B and C, and was therefore chosen for the transfection experiments.

5 The cell line PO.HTR has been deposited in accordance with the Budapest Treaty at the Institute Pasteur Collection Nationale De Cultures De Microorganismes, 28, Rue de Docteur Roux, 75724 Paris France, and has accession number I-1117.

10 This methodology is adaptable to secure other cell lines which are variants of a cell type which normally presents at least one of the four recognized P815 tumor antigens, i.e., antigens A, B, C and D, where the variants present none of antigens A, B and C. P1.HTR is a mastocytoma cell line, so it will be seen that the protocol 15 enables the isolation of biologically pure mastocytoma cell lines which express none of P815 antigens A, B and C, but which are highly transfectable. Other tumor types may also be screened in this fashion to secure desired, biologically 20 pure cell lines. The resulting cell lines should be at least as transfectable with foreign DNA as is P1.HTR, and should be selected so as to not express a specific antigen.

#### Example 2

25 Previous work reported by DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988) the disclosure of which is incorporated by reference herein had shown the efficacy of using cosmid library transfection to recover genes coding for tum antigens.

30 Selective plasmid and genomic DNAs of P1.HTR were prepared, following Wölfel et al., Immunogenetics 26: 178-187 (1987). The transfection procedure followed Corsaro et al., Somatic Cell Molec. Genet 7: 603-616 (1981), with some modifications. Briefly, 60 µg of cellular DNA and 3 µg of DNA of plasmid pHMR272, described by Bernard et al., Exp. Cell. Biol. 158: 237-243 (1985) were mixed. This plasmid 35 confers hygromycin resistance upon recipient cells,

and therefore provides a convenient way to screen for transfectants. The mixed DNA was combined with 940 ul of 1 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, and 310 ul 1M CaCl<sub>2</sub>. The solution was added slowly, and under constant agitation 5 to 1.25 ml of 50 mM Hepes, 280 mM NaCl, 1.5 mM Na<sub>2</sub>HPO<sub>4</sub>, adjusted to pH 7.1 with NaOH. Calcium phosphate - DNA precipitates were allowed to form for 30-45 minutes at room temperature. Following this, fifteen groups of PO.HTR 10 cells (5x10<sup>6</sup> per group) were centrifuged for 10 minutes at 400xg. Supernatants were removed, and pellets were resuspended directly into the medium containing the DNA 15 precipitates. This mixture was incubated for 20 minutes at 37°C, after which it was added to an 80 cm<sup>2</sup> tissue culture flask containing 22.5 ml DMEM, supplemented with 10% fetal calf serum. After 24 hours, medium was replaced. Forty-eight hours after transfection, cells were collected and 20 counted. Transfected cells were selected in mass culture using culture medium supplemented with hygromycin B (350 ug/ml). This treatment selected cells for hygromycin resistance.

For each group, two flasks were prepared, each containing 8x10<sup>6</sup> cells in 40 ml of medium. In order to estimate the number of transfectants, 1x10<sup>6</sup> cells from each 25 group were plated in 5 ml DMEM with 10% fetal calf serum (FCS), 0.4% bactoagar, and 300 ug/ml hygromycin B. The colonies were then counted 12 days later. Two independent determinations were carried out and the average taken. This was multiplied by 5 to estimate the number of 30 transfectants in the corresponding group. Correction had to be made for the cloning efficiency of P815 cells, known to be about 0.3.

#### Example 3

Eight days after transfection as described in example 2, supra, antibiotic resistant transfectants were separated 35 from dead cells, using density centrifugation with Ficoll-

Paqu. These cells were maintained in non-selective medium for 1 or 2 days. The cells were plated in 96 well microplates (round bottom), at 30 cells/microwell in 200  $\mu$ l of culture medium. Anywhere from 100-400 microwells were prepared, depending on the number of transfectants prepared. Agar colony tests gave estimates of 500-3000. After 5 days, the wells contained about  $6 \times 10^4$  cells and replicate plates were prepared by transferring 1/10 of the wells to microplates which were then incubated at 30°C. One day later, master plates were centrifuged, medium removed, and 750 CTLs against P815 antigen A (CTL.P1:5) were added to each well together with  $10^6$  irradiated syngeneic feeder spleen cells in CTL culture medium containing 40 U/ml recombinant human IL-2, and HAT medium to kill stimulator cells. Six days later, plates were examined visually to identify wells where CTLs had proliferated. Where plates showed proliferating microcultures, aliquots of 100  $\mu$ l of the wells were transferred to another plate containing  $^{51}\text{Cr}$  labeled P1.HTR target cells ( $2 \times 10^3$  -  $4 \times 10^3$  per well), and chromium release was measured after 4 hours. Replicate microcultures corresponding to those showing high CTL activity were expanded and cloned by limited dilution in DMEM with 10% FCS. Five days later, about 200 clones were collected and screened with the CTL.P1:5 cell line, described supra, in a visual lysis assay. See figure 1A for these results.

In these experiments, three of the fifteen groups of transfectants yielded a few positive microcultures. These microcultures were tested for lytic activity against P1.HTR, as described supra. Most of the microcultures where proliferation was observed showed lytic activity. This activity was well above background, as shown in figure 1B. This figure summarizes data wherein two groups of cells (groups "5" and "14"), 400 and 300 microwells were seeded with 30 hygromycin resistant transfected cells. Amplification and duplication of the microcultures was followed by addition of anti-A CTL P1:5. Six days later,

lytic activity against P1.HTR was tested. In the figure, each point represents lytic activity of a single microculture.

5 Duplicate microcultures corresponding to several positive wells were subcloned, and more than 1% of the subclones were found to be lysed by anti-A CTL. Thus, three independent transfectants expressing P815A were obtained from 33,000 hygromycin resistant transfectants. One of these lines, referred to hereafter as P1A.T2 was 10 tested further.

The relevant antigen profile of P1A.T2 is shown in figure 2, this being obtained via anti-CTL assays of the type described supra.

**Example 4**

15 The CTL assays carried out for P1A.T2 demonstrated that it presented antigen A ("P815A"), and therefore had received the gene from P1.HTR. To that end, this cell line was used as a source for the gene for the antigen precursor in the following experiments.

20 Prior work had shown that genes coding for tum-antigens could be recovered directly from transfectants obtained with a cosmid library. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988). This procedure was followed for recovery of the P815 gene.

25 Total genomic DNA of P1A.T2 was partially digested with restriction endonuclease Sau 3A1, and fractionated by NaCl density gradient ultracentrifugation to enrich for 35-50 kb DNA fragments, following Grosveld et al., Gene 10: 6715-6732 (1982). These fragments were ligated to cosmid 30 arms of C2RB, described by Bates et al., Gene 26: 137-146 (1983), the disclosure of which is incorporated by reference. These cosmid arms had been obtained by cleavage with SmaI and treatment with calf intestinal phosphatase, followed by digestion with BamHI. Ligated DNA was packaged 35 into lambda phage components, and titrated on *E. coli* ED 8767, following Grosveld et al., supra. Approximately  $9 \times 10^5$

ampicillin resistant colonies were obtained per microgram of DNA insert.

The cosmid groups were amplified by mixing 30,000 independent cosmids with 2 ml of ED 8767 in 10 mM MgCl<sub>2</sub>, 5 incubated 20 minutes at 37°C, diluted with 20 ml of Luria Bertani ("LB") medium, followed by incubation for one hour. This suspension was titrated and used to inoculate 1 liter of LB medium in the presence of ampicillin (50 ug/ml). At 10 a bacterial concentration of 2x10<sup>8</sup> cells/ml (OD<sub>600</sub>=0.8), a 10 ml aliquot was frozen, and 200 ug/ml chloramphenicol was added to the culture for overnight incubation. Total cosmid DNA was isolated by alkaline lysis procedure, and purified on CsCl gradient.

15 In these experiments, a library of 650,000 cosmids was prepared. The amplification protocol involved the use of 21 groups of approximately 30,000 cosmids.

Example 5

20 Using the twenty-one groups of cosmids alluded to supra, (60 ug) and 4 ug of pHMR272, described supra, groups of 5x10<sup>6</sup> P0.HTR cells were used as transfectant hosts. Transfection was carried out in the same manner as 25 described in the preceding experiments. An average of 3000 transfectants per group were tested for antigen presentation, again using CTL assays as described. One group of cosmids repeatedly yielded positive transfectants, at a frequency of about 1/5,000 drug resistant transfectants. The transfectants, as with P1A.T2, also showed expression of both antigen A and B. The pattern of expression of transfectant P1A.TC3.1 is shown in figure 2,

30 Example 6

As indicated in Example 5, supra, three independent 35 cosmid transfected cells presenting P815A antigen were isolated. The DNA of these transfectants was isolated and packaged directly with lambda phage extracts, following DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278

(1988). The resulting product was titrated on E. coli ED 8767 with ampicillin selection, as in Example 5. Similarly, amplification of the cosmids and transfection followed Example 5, again using PO.HTR.

5 High frequencies of transfection were observed, as described in Table 1, which follows:

Table 1. Transfer of the expression of antigen P815A by cosmids obtained by direct packaging

10	Transfectant obtained with the cosmid library	No. of cosmids obtained by direct packaging of 0.5 µg of DNA	No. of transfectants expressing P815A / no. of Hm <sup>b</sup> transfectants
15	TC3.1	32	87/192
	TC3.2	32000	49/384
	TC3.3	44	25/72

20 The cosmids were analyzed with restriction enzymes and it was found that directly packaged transfectant P1A.TC3.1 contained 32 cosmids, 7 of which were different. Each of these 7 cosmids was transfected into PO.HTR, in the manner described supra, and again, following the protocols described above, transfectants were studied for presentation of P815A. Four of the cosmid transfectants showed P815A presentation and, as with all experiments described herein, P815B was co-expressed.

25 Of the four cosmids showing presentation of the two antigens, cosmid C1A.3.1 was only 16.7 kilobases long, and was selected for further analysis as described infra.

30 The cosmid C1A.3.1 was subjected to restriction endonuclease analysis, yielding the map shown in Figure 3.

35 All EcoRI fragments were transfected, again using the above described protocols, and only the 7.4 kilobase fragment produced a transfectant that anti-A CTLs could lyse. Similar experiments were carried out on the PstI

fragments, and only a 4.1 kb fragment fully contained within the 7.4 kb EcoRI fragment produced lysable transfectants.

5        This fragment (i.e., the 4.1 kb PstI fragment), was digested with SmaI, giving a 2.3 kb fragment which also yielded host cells presenting antigens A and B after transfection. Finally, a fragment 900 bases long, secured with SmaI/XbaI, also transferred expression of the precursors of these two antigens, i.e., the transfected 10      host cell presented both antigen A and antigen B.

Example 7

15       The 900 base fragment described above was used as a probe to detect the expression of the P815A gene in parent cell line P1.HTR. To accomplish this, total cellular RNA was first isolated using the guanidine-isothiocyanate procedure of Davis et al., Basic Methods In Molecular Biology (Elsevier Science Publishing Co, New York) (1986). The same reference was the source of the method used to 20      isolate and purify polyA<sup>+</sup> mRNA using oligodT cellulose column chromatography.

25       Samples were then subjected to Northern Blot analysis. RNA samples were fractionated on 1% agarose gels containing 0.66 M formaldehyde. The gels were treated with 10xSSC (SSC: 0.15 M NaCl; 0.015 M sodium citrate, pH 7.0) for 30 minutes before overnight blotting on nitrocellulose membranes. These were baked for two hours at 80°C, after which the membranes were prehybridized for 15 minutes at 60°C in a solution containing 10% dextran sulfate, 1% SDS and 1M NaCl. Hybridization was then carried out using 30      denatured probe (the 900 base fragment), together with 100 ug/ml salmon sperm DNA.

When this protocol was carried out using P1.HTR poly A<sup>+</sup> RNA, a band of 1.2 kb and two fainter bands were identified, as shown in Figure 4, lane 1 (6 ug of the RNA).

35       The same probe was used to screen a cDNA library, prepared from poly-A<sup>+</sup> RNA from the cell line. This yielded

a clone with a 1kb insert, suggesting a missing 5' end. The Northern blots for the cDNA are not shown.

5 Hybridization experiments in each case were carried out overnight at 60°C. The blots were washed twice at room temperature with 2xSSC and twice at 60°C with 2xSSC supplemented with 1% SDS.

10 The foregoing experiments delineated the DNA expressing the P815A antigen precursor sufficiently to allow sequencing, using the well known Sanger dideoxy chain termination method. This was carried out on clones generated using a variety of restriction endonucleases and by specific priming with synthetic oligonucleotide primers. The results for exons of the gene are set forth in SEQUENCE ID NO: 4.

15 **Example 8**

The Northern analysis described supra suggested that the 5' end of the cDNA was missing. To obtain this sequence, cDNA was prepared from P1.HTR RNA using a primer corresponding to positions 320-303. The sequence was then 20 amplified using the polymerase chain reaction using a 3' primer corresponding to positions 286-266 and a 5' primer described by Frohman et al., Proc. Natl. Acad. Sci. USA 85: 8998-9002 (1988). A band of the expected size (270 bases) was found, which hybridized to the 900 bp SmaI/XbaI 25 fragment described supra on a Southern blot. Following cloning into m13tg 130 and tg 131, the small, 270 bp fragment was sequenced. The sequence is shown in SEQUENCE ID NO: 1.

**Example 9**

30 Following the procurement of the sequences described in Examples 7 and 8 and depicted in SEQ ID NO: 4, a 5.7 kb region of cosmid C1A.3.1 was sequenced. This fragment was known to contain the 900 base fragment which expressed P815A in transfectants. This experiment permitted

delineation of introns and exons, since the cosmid is genomic in origin.

The delineated structure of the gene is shown in figure 5. Together with SEQ ID NO: 4, these data show that 5 the gene for the antigen precursor, referred to as "P1A" hereafter, is approximately 5 kilobases long and contains 10 3 exons. An ORF for a protein of 224 amino acids starts in exon 1, ending in exon 2. The 900 base pair fragment which transfers expression of precursors for antigens A and B only contains exon 1. The promoter region contains a CAAT 15 box, as indicated in SEQ ID NO: 1, and an enhancer sequence. This latter feature has been observed in promoters of most MHC class I genes, as observed by Geraghty et al., J. Exp. Med 171: 1-18 (1990); Kimura et al., Cell 44: 261-272 (1986).

A computer homology search was carried out, using 20 program FASTA with K-triple parameters of 3 and 6, as suggested by Lipman et al., Science 227: 1435-1441 (1985), and using Genbank database release 65 (October 1990). No 25 homology was found except for a stretch of 95 bases corresponding to part of an acid region coded by exon 1 (positions 524-618), which is similar to sequences coding for acidic regions in mouse nucleolar protein NO38/B23, as described by Bourbon et al., Mol. Biol. 200: 627-638 (1988), and Schmidt-Zachmann et al., Chromosoma 96: 417-426 (1988). Fifty six of 95 bases were identical. In order to 30 test whether these homologies were the reason for cross hybridizing, experiments were carried out using a mouse spleen cDNA library screened with the 900 base fragment. cDNA clones corresponding closely to the sizes of the cross hybridizing bands were obtained. These were partially 35 sequenced, and the 2.6 kb cDNA was found to correspond exactly to reported cDNA sequence of mouse nucleolin, while the 1.5 kb cDNA corresponded to mouse nucleolar protein NO38/B23.

Analysis of the nucleotide sequence of the gene, referred to as "P1A" hereafter, suggests that its coded

product has a molecular mass of 25 kd. Analysis of the SEQUENCE ID NO: 4 shows a potential nuclear targeting signal at residues 5-9 (Dingwall et al., Ann. Rev. Cell Biol. 2: 367-390 (1986)), as well as a large acidic domain at positions 83-118. As indicated supra, this contains the region of homology between P1A and the two nucleolar proteins. A putative phosphorylation site can be found at position 125 (serine). Also, a second acidic domain is found close to the C-terminus as an uninterrupted stretch of 14 glutamate residues. A similar C-terminal structure has been found by Kessel et al. Proc. Natl. Acad. Sci. USA 84: 5306-5310 (1987), in a murine homeodomain protein having nuclear localization.

In studies comparing the sequence of gene P1A to the sequences for P91A, P35B and P198, no similarities were found, showing that P1A is indicative of a different class of genes and antigens.

#### Example 10

With the P1A probe and sequence in hand, investigations were carried out to determine whether the gene present in normal tissue was identical to that expressed by the tumor. To do this, phage libraries were prepared, using lambda zapII 10 and genomic DNA of DBA/2 murine kidney cells. P1A was used as a probe. Hybridization conditions were as described supra, and a hybridizing clone was found. The clone contained exons one and two of the P1A gene, and corresponded to positions -0.7 to 3.8 of figure 5. Following localization of this sequence, PCR amplification was carried out to obtain the sequence corresponding to 3.8 to 4.5 of figure 5.

Sequence analysis was carried out, and no differences were found between the gene from normal kidneys and the P1A gene as obtained from the P815 tumor cells.

In further experiments, the gene as found in DBA/2 kidney cells was transfected into PO.HTR, as described supra. These experiments, presented pictorially in figure

6, showed that antigens A and B were expressed as efficiently by the kidney gene isolated from normal P815 cells as with the P1A gene isolated from normal kidney cells.

5 These experiments lead to the conclusion that the gene coding for the tumor rejection antigen precursor is a gene that does not result from a mutation; rather, it would appear that the gene is the same as one present in normal cells, but is not expressed therein. The ramifications of 10 this finding are important, and are discussed infra.

In studies not elaborated upon herein, it was found that variants of the gene were available. Some cells were "P1AB<sup>+</sup>", rather than the normal "P1A". The only difference between these is a point mutation in exon 1, with the 18th 15 triplet coding for Ala in the variant instead of Val.

#### Example 11

Additional experiments were carried out with other 20 cell types. Following the protocols described for Northern blot hybridizations supra, RNA of normal liver and spleen cells was tested to determine if a transcript of the P1A gene could be found. The Northern blot data are presented in figure 4 and, as can be seen, there is no evidence of expression.

The murine P815 cell line from which P1A was isolated 25 is a mastocytoma. Therefore, mast cell lines were studied to determine if they expressed the gene. Mast cell line MC/9, described by Nabel et al., Cell 23: 19-28 (1981), and short term cultures of bone marrow derived mast cells were tested in the manner described supra (Northern blotting), but no transcript was found. In contrast when a BALB/C 30 derived IL-3 dependent cell line L138.8A (Hültner et al., J. Immunol. 142: 3440-3446 (1989)) was tested, a strong signal was found. The mast cell work is shown in figure 4.

It is known that both BALB/C and DBA/2 mice share H-2<sup>d</sup> 35 haplotype, and thus it was possible to test sensitivity to lysis using the CTLs described supra. Figure 7 shows these

results, which essentially prove that anti-A and anti-B CTLs lysed the cells strongly, whereas anti-C and anti-D lines did not.

5 Further tests were carried out on other murine tumor cell lines, i.e., teratocarcinoma cell line PCC4 (Boon et al., Proc. Natl. Acad. Sci. USA 74: 272-275 (1977), and leukemias LEC and WEHI-3B. Expression could not be detected in any of these samples.

**Example 12**

10 The actual presentation of the P1A antigen by MHC molecules was of interest. To test this, cosmid C1A.3.1 was transfected into fibroblast cell line DAP, which shows phenotype H-2<sup>k</sup>. The cell lines were transfected with genes expressing one of the K<sup>d</sup>, D<sup>d</sup>, and L<sup>d</sup> antigen. Following 15 transfection with both the cosmid and the MHC gene, lysis with CTLs was studied, again as described supra. These studies, summarized in Table 2, show that L<sup>d</sup> is required for presentation of the P1A antigens A and B.

20 **Table 2. H-2-restriction of antigens P815A and P815B**

	Recipient cell*	No. of clones lysed by the CTL/no. of HmB' clones*	
		CTL anti-A	CTL anti-B
25	DAP (H-2 <sup>k</sup> )	0/208	0/194
	DAP + K <sup>d</sup>	0/165	0/162
	DAP + D <sup>d</sup>	0/157	0/129
	DAP + L <sup>d</sup>	25/33	15/20

30 \*Cosmid C1A.3.1 containing the entire P1A gene was transfected in DAP cells previously transfected with H-2<sup>d</sup> class I genes as indicated.

\*Independent drug-resistant colonies were tested by lysis by anti-A or anti-B CTL in a visual assay.

The observation that one may associate presentation of a tumor rejection antigen with a particular MHC molecule was confirmed in experiments with human cells and HLA molecules, as elaborated upon infra.

5       Example 13

Using the sequence of the P1A gene as well as the amino acid sequence derivable therefrom, antigenic peptides which were A<sup>+</sup> B<sup>+</sup> (i.e., characteristic of cells which express both the A and B antigens), and those which are AB<sup>+</sup> were identified. The peptide is presented in SEQ ID NO: 10 26. This peptide when administered to samples of PO.HTR cells in the presence of CTL cell lines specific to cells presenting it, led to lysis of the PO.HTR cells, lending support to the view that peptides based on the product 15 expressed by the gene can be used as vaccines.

Example 14

The human melanoma cell line referred to hereafter as MZ2-MEL is not a clonal cell line. It expresses four stable antigens recognized by autologous CTLs, known as 20 antigens "D, E, F, and A". In addition, two other antigens "B" and "C" are expressed by some sublines of the tumor. CTL clones specific for these six antigens are described by Van den Eynde et al., Int. J. Canc. 44: 634-640 (1989). Among the recognized subclones of MZ2-MEL are MEL.43, 25 MEL3.0 and MEL3.1. (Van den Eynde et al., supra). Cell line MEL3.1 expresses antigen E, as determined by CTL studies as described for P815 variants, supra, so it was chosen as a source for the nucleic acid sequence expressing the antigen precursor.

30       In isolating the pertinent nucleic acid molecule for a tumor rejection antigen precursor, the techniques developed supra, showed that a recipient cell is needed which fulfills two criteria: (i) the recipient cell must not express the TRAP of interest under normal conditions,

and (ii) it must express the relevant class I HLA molecule. Also, the recipient cell must have a high transfection frequency, i.e., it must be a "good" recipient.

5 In order to secure such a cell line, the clonal subline MEL3.1 was subjected to repeated selection with anti-E CTL 82/30 as described by Van den Eynde, supra. The repeated cycles of selection led to isolation of subclone MZ2-MEL-2.2 isc E. This subclone is also HPRT, (i.e., sensitive to HAT medium:  $10^4$  M hypoxanthine,  $3.8 \times 10^{-7}$  aminopterine,  $1.6 \times 10^{-5}$  M 2-deoxythymidine). The subclone 10 is referred to as "MEL-2.2" for simplicity hereafter.

Example 15

15 The genomic DNA of MEL3.0 was prepared following Wölfel et al., Immunogenetics 26: 178-187 (1987), the disclosure of which is incorporated by reference. The plasmid pSVtkneo $\beta$ , as described by Nicolas et al., Cold Spring Harb., Conf. Cell Prolif. 10: 469-485 (1983) confers geneticin resistance, so it can be used as a marker for cotransfection, as it was in this experiment.

20 Following a procedure similar but not identical to that of Corsaro et al., Somatic Cell Molec. Genet 7: 603-616 (1981), total genomic DNA and the plasmid were 25 cotransfected. The genomic DNA (60  $\mu$ g) and plasmid DNA (6  $\mu$ g) were mixed in 940  $\mu$ l of 1 mM Tris·HCl (pH 7.5), 0.1 mM EDTA, after which 310  $\mu$ l of 1M CaCl<sub>2</sub> was added. This solution was slowly added, under constant agitation, to 1.25 ml of 2xHBS (50 mM HEPES, 280 mM NaCl 1.5 mM Na<sub>2</sub>HPO<sub>4</sub>, 30 adjusted to pH 7.1 with NaOH). The calcium phosphate DNA precipitates were allowed to form for 30-45 minutes at room 35 temperature, after which they were applied to 80 cm<sup>2</sup> tissue culture flasks which had been seeded 24 hours previously with  $3 \times 10^6$  MEL2.2 cells, in 22.5 ml of melanoma culture medium (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal calf serum. After 24 hours, the medium was replaced. Forty eight hours after transfection, the cells

were harvested and seeded at  $4 \times 10^6$  cells per  $80 \text{ cm}^2$  flask in melanoma culture medium supplemented with 2 mg/ml of geneticin. The geneticin serves as a selection marker.

**Example 16**

5        Thirteen days after transfection, geneticin-resistant colonies were counted, harvested, and cultured in nonselective medium for 2 or 3 days. Transfected cells were then plated in 96-well microplates at 200 cells/well in 200  $\mu\text{l}$  of culture medium with 20% fetal calf serum (FCS) 10      in order to obtain approximately 30 growing colonies per well. The number of microcultures was aimed at achieving redundancy, i.e., such that every independent transfectant should be represented at least four times.

15      After 10 days, wells contained approximately  $6 \times 10^4$  cells. These cells were detached, and 1/3 of each microculture was transferred to a duplicate plate. After 6 hours, i.e., after readherence, medium was removed and 1500 anti-E CTL (CTL 82/30), were added to each well in 100  $\mu\text{l}$  of CTL culture medium with 35 U/ml of IL-2. One day 20      later, the supernatant (50  $\mu\text{l}$ ) was harvested and examined for TNF concentration, for reasons set forth in the following example.

**Example 17**

25      The size of the mammalian genome is  $6 \times 10^6$  kb. As the average amount of DNA integrated in each drug-resistant transfectant was expected to be about 200 kb, a minimum of 30,000 transfectants would need to be examined to ascertain whether antigen E had been transfected. Prior work with 30      murine cells had shown that when a CTL stimulation assay was used, groups containing only 3% of cells expressing the antigen of interested could be identified. This should reduce the number of assays by a factor of 30. While an anti-E CTL assay, as described supra, in mixed  $\text{E}^+/\text{E}$  cells 35      was helpful, it was not sufficient in that consistent results could not be obtained.

As a result, an alternative test was devised. Stimulation of CTLs was studied by release of tumor necrosis factor ("TNF") using well known methodologies which need not be repeated here. As described in Example 5 15, 1500 CTL 82/30 cells had been added per well of transfectants. These CTLs were collected 6 days after stimulation. As indicated supra, after 1/3 of the cells in each well had been removed and the remaining 2/3 ( $4 \times 10^4$ ) had readhered, the CTLs and IL-2 were added thereto. The 50  $\mu$ l 10 of supernatant was removed 24 hours later and transferred to a microplate containing  $3 \times 10^4$  W13 (WEHI-164 clone 13; Espevik et al., J. Immunol. Meth. 95: 99-105 (1986)) cells in 50  $\mu$ l of W13 culture medium (RPMI-1640, supplemented with L-arginine (116 mg/l), L-asparagine (36 mg/l), L- 15 glutamine (216 mg/l), and 10% FCS supplemented with 2  $\mu$ g of actinomycin D at 37°C in an 8% CO<sub>2</sub> atmosphere. The cell line W13 is a mouse fibrosarcoma cell line sensitive to TNF. Dilutions of recombinant TNF- $\beta$  in RPMI 1640 were added to target cell controls.

20 The W13 cultures were evaluated after 20 hours of incubation, and dead cell percentage was measured using an adaptation of the colorimetric assay of Hansen et al., J. Immunol. Meth. 119: 203-210 (1989). This involved adding 50 ml of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl 25 tetrazolium bromide at 2.5 mg/ml in PBS, followed by two hours of incubation at 37°C. Dark blue formazan crystals were dissolved by adding 100  $\mu$ l of lysis solution (1 volume N,N dimethyl formamide mixed at 37°C with two volumes of water containing 30% (w/v) sodium dodecyl sulphate, at pH 30 4.7 from 1.6% acetic acid and 2.5% 1N HCl). Plates were incubated at 37°C overnight, and ODs were taken at 570 nm using 650 nm as control. Dead cell percentage was determined via the formula:

$$35 \quad 100 \times 1 - \frac{100 - (\text{OD}_{570} \text{ sample well})}{\text{OD}_{570} \text{ well + medium}}$$

following Espevik et al., J. Immunol. Meth. 95: 99-105 (1986). The results showed that even when the ratio of E<sup>+</sup>/E cells was as low as 1/45, significant production of TNF was observed, thus showing active CTLs. This led to 5 the decision to test the drug resistant transfectants in groups of 30.

Example 18

Cells were tested for TNF production as discussed in Example 17, supra. A total of 100 groups of E cells (4x10<sup>6</sup> cells/group) were tested following transfection, and 7x10<sup>4</sup> 10 independent geneticin resistant transfectants were obtained, for an average of 700 per group. Only one group of transfected cells led to a microculture which caused anti-E antigen CTL clone 82/30 to produce TNF. Of 300 15 clones tested, 8 were positive. These clones were then tested for lysis by anti-E CTL, using the standard <sup>51</sup>Cr release assay, and were found to be lysed as efficiently as the original E<sup>+</sup> cell line. The transfectant E.T1, discussed herein, had the same lysis pattern as did MEL2.2 for CTLs 20 against antigens B,C,D and F.

The fact that only one transfectant presented the antigen out of 70,000 geneticin resistance transfectants may at first seem very low, but it is not. The work described supra for P815 showed an average frequency of 25 1/13,000. Human DNA recipient MEL2.2 appears to integrate 5 times less DNA than P1.HTR.

Example 19

Once transfectant E.T1 was found, analysis had to 30 address several questions including whether an E<sup>+</sup> contaminant of the cell population was the cause. The analysis of antigen presentation, described supra, shows that E.T1 is B<sup>-</sup> and C<sup>-</sup>, just like the recipient cell MEL2.2. It was also found to be HPRT<sup>-</sup>, using standard selection

procedures. All E<sup>+</sup> cells used in the work described herein, however, were HPRT<sup>+</sup>.

It was also possible that an E<sup>+</sup> revertant of MEL2.2 was the source for E.T1. To test this, the observation by Perucho et al., Cell 22: 309-317 (1980), that cotransfected sequences usually integrate together at a single location of recipient genome was employed. If antigen E in a transfectant results from cotransfection with pSVtkneo $\beta$ , then sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneo $\beta$  sequences. Wölfel et al., supra, has shown this to be true. If a normally E cell is transfected with pSVtkneo $\beta$ , then sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneo $\beta$  sequences. If a normally E<sup>+</sup> cell transfected with pSVtkneo $\beta$  is E.T1, however, "co-deletion" should not take place. To test this, the transfectant E.T1 was subjected to immunoselection with 82/30, as described supra. Two antigen loss variants were obtained, which resisted lysis by this CTL. Neither of these had lost geneticin resistance; however, Southern blot analysis showed loss of several neo<sup>r</sup> sequences in the variants, showing close linkage between the E gene and neo<sup>r</sup> gene in E.T1, leading to the conclusion that E.T1 was a transfectant.

25 **Example 20**

The E<sup>+</sup> subclone MZ2-MEL 43 was used as a source of DNA for preparation of a cosmid library. This library of nearly 700,000 cosmids was transfected into MZ2-MEL 2.2 cells, following the cosmid transfection protocols described supra.

30 By packaging the DNA of cosmid transfectants directly into lambda phage components, it is sometimes possible to retrieve cosmids that contain the sequences of interest. This procedure was unsuccessful here, so rescue of the transfectant sequence was accomplished by ligating DNA of

the transfectant to appropriate restriction fragments of cosmid vector pTL6. This was tried with two transfectants and was successful with one of them. One cosmid, referred to as B3, was recovered from this experiment, and subjected to restriction endonuclease digestion via XmaI, or by BamHI digestion of a large, 12 kb XmaI transfected fragment. The fragments were cloned into vector pTZ 18R, and then transfected into MEL2.2. Again, TNF production was the measure by which successful transfection was determined. The experiments led to the determination of a gene sequence capable of transfecting antigen E on the 12 kb XmaI fragment, and then on the 2.4 kb fragment of BamHI digestion of the 12 kb segment.

The 2.4 kb fragment hybridizes with a 2.4 kb fragment from MZ2-MEL and with a T cell clone of patient MZ-2, as determined by Southern Blots (BamHI digested DNA). The band is absent from E antigen loss variants of MZ2-MEL, as seen in SEQ ID NO: 7.

The sequence for the E antigen precursor gene has been determined, and is presented herein:

Example 21

After the 2.4 kb genomic segment had been identified, studies were carried out to determine if an "E<sup>+</sup>" subline expressed any homologous DNA. Cell line MZ2-MEL 3.0 was used as a source, and a cDNA library was prepared from its mRNA, using art known techniques. The 2.4 kb segment was used as a probe, and an mRNA of about 1.8 kb was identified as homologous, using Northern blot analysis. When cDNA was screened, clones were obtained showing almost complete identity to parts of the 2.4 kb fragment. Two exons were thus identified. An additional exon was located upstream of these, via sequencing segments of cosmid B3 located in front of the 2.4 kb BamHI fragment. The gene extends over about 4.5 kb, as shown in Figure 8. The starting point of the transcribed region was confirmed using PCR for the 5' end of the cDNA. The three exons comprise 65, 73, and 1551

base pairs. An ATG is located at position 66 of exon 3, followed by a 927 base pair reading frame.

Example 22

5 To determine if smaller segments of the 2.4 kb fragment could transfer the expression of antigen E, smaller pieces corresponding to the larger gene were prepared, using art recognized techniques, and transferred into E cells. Figure 8 shows the boundaries of the three segments.

10 Transfer of antigen expression in this manner indicates that the gene codes for the antigen precursor, rather than coding for a protein which activates the antigen.

Example 23

15 The probing of cDNA described supra revealed, surprisingly, two different but closely related cDNAs. These cDNAs, when tested, did not transfer expression of antigen E, but they do show substantial homology to the first cDNA segment. The three segments, appear to indicate 20 a newly recognized family of genes, referred to as "MAGE" for "melanoma antigen". In Figure 9, MAGE-1" directs expression of the antigen from MZ2 cells. Portions of the third exon of each gene are presented in Figure 9. The second and third sequences are more closely related to each 25 other than the first (18.1 and 18.9% difference compared to the first; 12% with each other). Out of 9 cDNA clones obtained, three of each type were obtained, suggesting equal expression. "MAGE" as used hereafter refers to a family of molecules, and the nucleic acids coding for them. 30 These nucleic acids share a certain degree of homology and are expressed in tumor cells including several types of human tumor cells as well as in human tumors. The family is referred to as "MAGE" because the first members were identified in human melanoma cells. As the experiments 35 which follow indicate, however, the members of the MAGE

family are not at all restricted to melanoma tumors; rather, MAGE refers to a family of tumor rejection antigen precursors and the nucleic acid sequences coding therefore. The antigens resulting therefrom are referred to herein as 5 "MAGE TRAs" or "melanoma antigen tumor rejection antigens"

Example 24

Experiments with mouse tumors have demonstrated that new antigens recognized by T cells can result from point mutations that modify active genes in a region that codes 10 for the new antigenic peptide. New antigens can also arise from the activation of genes that are not expressed in most normal cells. To clarify this issue for antigen MZ2-E, the MAGE-1 gene present in the melanoma cells was compared to that present in normal cells of patient MZ2. Amplification 15 by polymerase chain reaction (PCR) of DNA of phytohemagglutinin-activated blood lymphocytes using primers surrounding a 1300 bp stretch covering the first half of the 2.4 kb fragment was carried out. As expected, a PCR product was obtained whereas none was obtained with 20 the DNA of the E variant. The sequence of this PCR product proved identical to the corresponding sequence of the gene carried by the E<sup>+</sup> melanoma cells. Moreover, it was found that antigen MZ2-E was expressed by cells transfected with 25 the cloned PCR product. This result suggests that the activation of a gene normally silent is responsible for the appearance of tumor rejection antigen MZ2-E.

Example 25

In order to evaluate the expression of gene MAGE-1 by 30 various normal and tumor cells, Northern blots were hybridized with a probe covering most of the third exon. In contrast with the result observed with human tumor cell line MZ2-MEL 3.0, no band was observed with RNA isolated 35 from a CTL clone of patient MZ2 and phytohemagglutinin-activated blood lymphocytes of the same patient. Also negative were several normal tissues of other individuals

(Figure 10 and Figure 11). Fourteen melanoma cell lines of other patients were tested. Eleven were positive with bands of varying intensities. In addition to these cultured cell lines, four samples of melanoma tumor tissue were analyzed. Two samples, including a metastasis of patient MZ2, proved positive, excluding the possibility that expression of the gene represented a tissue culture artefact. A few tumors of other histological types, including lung tumors were tested. Most of these tumors were positive (Figures 10 and 11). These results indicated that the MAGE gene family is expressed by many melanomas and also by other tumors. However, they provided no clear indication as to which of genes MAGE-1, 2 or 3 were expressed by these cells, because the DNA probes corresponding to the three genes cross-hybridized to a considerable extent. To render this analysis more specific, PCR amplification and hybridization with highly specific oligonucleotide probes were used. cDNAs were obtained and amplified by PCR using oligonucleotide primers corresponding to sequences of exon 3 that were identical for the three MAGE genes discussed herein. The PCR products were then tested for their ability to hybridize to three other oligonucleotides that showed complete specificity for one of the three genes (Figure 9). Control experiments carried out by diluting RNA of melanoma MZ2-MEL 3.0 in RNA from negative cells indicated that under the conditions used herein the intensity of the signal decreased proportionally to the dilution and that positive signals could still be detected at a dilution of 1/300. The normal cells (lymphocytes) that were tested by PCR were confirmed to be negative for the expression of the three MAGE genes, suggesting therefore a level of expression of less than 1/300<sup>th</sup> that of the MZ2 melanoma cell line (Figure 11). For the panel of melanoma cell lines, the results clearly showed that some melanomas expressed MAGE genes mage 1, 2 and 3 whereas other expressed only mage-2 and 3 (Figures 11 and 10). Some of the other tumors also

expressed all three genes whereas others expressed only MAGE-2 and 3 or only MAGE-3. It is impossible to exclude formally that some positive PCR results do not reflect the expression of one of the three characterized MAGE genes but 5 that of yet another closely related gene that would share the sequence of the priming and hybridizing oligonucleotides. It can be concluded that the MAGE gene family is expressed by a large array of different tumors and that these genes are silent in the normal cells tested to this 10 point.

Example 26

The availability of a sequence that transfects at high efficiency and efficiently expresses a TRAP made it possible to search for the associated major 15 histocompatibility complex (MHC) class I molecule. The class I specificities of patient MZ2 are HLA-A1, A29, B37, B44 and C6. Four other melanomas of patients that had A1 in common with MZ2 were cotransfected with the 2.4 kb fragment and pSVtkneo $\beta$ . Three of them yielded neo' 20 transfectants that stimulated TNF release by anti-E CTL clone 82/30, which is CD8+ (Figure 11). No E- transfectant was obtained with four other melanomas, some of which shared A29, B44 or C6 with MZ2. This suggests that the presenting molecule for antigen MZ2-E is HLA-A1. In 25 confirmation, it was found that, out of 6 melanoma cell lines derived from tumors of HLA-A1 patients, two stimulated TNF release by anti-E CTL clone 82/30 of patient MZ2. One of these tumor cell lines, MI13443-MEL, also showed high sensitivity to lysis by these anti-E CTL, 30 These two melanomas were those that expressed MAGE-1 gene (Figure 11). Eight melanomas of patients with HLA haplotypes that did not include A1 were examined for their sensitivity to lysis and for their ability to stimulate TNF release by the CTL. None was found to be positive. The 35 ability of some human anti-tumor CTL to lyse allogeneic tumors sharing an appropriate HLA specificity with the

5 original tumor has been reported previously (Darrow, et al., J. Immunol. 142: 3329 (1989)). It is quite possible that antigenic peptides encoded by genes MAGE 2 and 3 can also be presented to autologous CTL by HLA-A1 or other class I molecules, especially in view of the similar results found with murine tumors, as elaborated upon supra.

**Example 27**

10 As indicated supra, melanoma MZ2 expressed antigens F, D and A', in addition to antigen E. Following the isolation of the nucleic acid sequence coding for antigen E, similar experiments were carried out to isolate the nucleic acid sequence coding for antigen F.

15 To do this, cultures of cell line MZ2-MEL2.2, an E cell line described supra, were treated with anti-F CTL clone 76/6, in the same manner described for treatment with anti-E CTL clones. This resulted in the isolation of an F antigen loss variant, which was then subjected to several rounds of selection. The resulting cell line, "MZ2-MEL2.2.5" was completely resistant to lysis by anti-F CTLs, 20 yet proved to be lysed by anti-D CTLs.

Again, following the protocols set forth for isolation 25 of antigen -E precursor DNA, the F variant was transfected with genomic DNA from F<sup>+</sup> cell line MZ2-MEL3.0. The experiments yielded 90,000 drug resistant transfectants. These were tested for MZ2-F expression by using pools of 30 cells in the TNF detection assay elaborated upon supra. One pool stimulated TNF release by anti-F CTLs, and was cloned. Five of 145 clones were found to stimulate anti-F CTLs. Lysis assays, also following protocols described 30 supra, confirmed (i) expression of the gene coding for antigen F, and (ii) presentation of antigen F itself.

**Example 28**

Following identification of F<sup>+</sup> cell lines, the DNA 35 therefrom was used to transfect cells. To do this, a cosmid library of F<sup>+</sup> cell line MZ2-MEL.43 was prepared,

again using the protocols described supra. The library was divided into 14 groups of about 50,000 cosmids, and DNA from each group was transfected into MZ2-MEL2.2.5. Transfectants were then tested for their ability to stimulate TNF release from anti-F CTL clone 76/6. Of 14 groups of cosmids, one produced two independent transfectants expressing antigen F; a yield of two positives out of 17,500 geniticing resistant transfectants.

**Example 29**

The existence of a gene family was suggested by the pattern observed on the Southern blot (Figure 12). To do this, the 2.4 kb BamHI fragment, which transferred the expression of antigen MZ2-E, was labelled with  $^{32}\text{p}$  and used as a probe on a Southern Blot of BamHI digested DNA of E + cloned subclone MZ2-MEL2.2. Hybridization conditions included 50  $\mu\text{l}/\text{cm}^2$  of 3.5xSSC, 1xDenhardt's solution; 25 mM sodium phosphate buffer (pH 7.0), 0.5% SDS, 2mM EDTA, where the 2.4 kb probes had been labelled with  $[\alpha^{32}\text{p}]$ dCTP (2-3000 Ci/mole), at  $3 \times 10^6$  cpm/ml. Hybridization was carried out for 18 hours at 65°C. After this, the membranes were washed at 65°C four times for one hour each in 2xSSC, 0.1% SDS, and finally for 30 minutes in 0.1xSSC, 0.1% SDS. To identify hybridization, membranes were autoradiographed using Kodak X-AR film and Kodak X-Omatic fine intensifying screens.

In the following examples, whenever "hybridization" is referred to, the stringency conditions used were similar to those described supra. "Stringent conditions" as used herein thus refers to the foregoing conditions; subject to routine, art recognized modification.

**Example 30**

The cDNA coding for MAGE 4 was identified from a sample of the human sarcoma cell line LB23-SAR. This cell line was found to not express MAGE 1, 2 or 3, but the mRNA of the cell line did hybridize to the 2.4 kb sequence for

MAGE 1. To study this further, a cDNA library was prepared from total LB23-SAR mRNA, and was then hybridized to the 2.4 kb fragment. A cDNA sequence was identified as hybridizing to this probe, and is identified hereafter as  
5 MAGE 4.

Example 31

Experiments were carried out using PHA-activated lymphocytes from patient "MZ2", the source of the "MZ" cells discussed supra. An oligonucleotide probe which  
10 showed homology to MAGE 1 but not MAGE 2 or 3 was hybridized with a cosmid library derived from the PHA activated cells. The size of the hybridizing BamHI cosmid fragment, however, was 4.5 kb, thus indicating that the material was not mage 1; however, on the basis of homology  
15 to mage 1-4, the fragment can be referred to as "MAGE 5". The sequence of MAGE 5 is presented in SEQ ID NO: 16.

Example 32

Melanoma cell line LB-33-MEL was tested. Total mRNA from the cell line was used to prepare cDNA, which was then  
20 amplified with oligos CHO9: (ACTCAGCTCCTCCCAGATT) (SEQ ID NO: 53), and CHO10: (GAAGAGGGAGGGGCCAAG) (SEQ ID NO: 54). These oligos correspond to regions of exon 3 that are common to previously described mage 1, 2 and 3.

To do this, 1  $\mu$ g of RNA was diluted to a total volume  
25 of 20  $\mu$ l, using 2  $\mu$ l of 10x PCR buffer, 2  $\mu$ l of each of 10 mM dNTP, 1.2  $\mu$ l of 25 mM MgCl<sub>2</sub>, 1  $\mu$ l of an 80 mM solution of  
CHO9, described supra, 20 units of RNAsin, and 200 units of  
30 M-MLV reverse transcriptase. This was followed by  
incubation for 40 minutes at 42°C. PCR amplification  
followed, using 8  $\mu$ l of 10x PCR buffer, 4.8  $\mu$ l of 25 mM  
35 MgCl<sub>2</sub>, 1  $\mu$ l of CHO10, 2.5 units of *Thermus aquaticus*  
("Taq") polymerase, and water to a total volume of 100  $\mu$ l.  
Amplification was then carried out for 30 cycles (1 minute  
94°C; 2 minutes at 52°C, 3 minutes at 72°C). Ten  $\mu$ l of  
each reaction were then size fractionated on agarose gel,

followed by nitrocellulose blotting. The product was found to hybridize with oligonucleotide probe CH018 (TCTTGATCCTGGAGTCC) (SEQ ID NO: 55). This probe identified mage 1 but not mage 2 or 3. However, the 5 product did not hybridize to probe SEQ 4 (TTGCCAAGATCTCAGGAA) (SEQ ID NO: 56). This probe also binds mage 1 but not 2 and 3. This indicated that the PCR product contained a sequence that differed from MAGE 1, 2 and 3. Sequencing of this fragment also indicated 10 differences with respect to MAGE 4 and 5. These results indicate a sequence differing from previously identified MAGE 1, 2, 3, 4 and 5, and is named MAGE 6.

Example 33

In additional experiments using cosmid libraries from 15 PHA-activated lymphocytes of MZ2, the 2.4 kb MAGE 1 fragment was used as a probe and isolated a complementary fragment. This clone, however, did not bind to oligonucleotides specific for MAGE 1, 2, 3 or 4. The sequence obtained shows some homology to exon 3 of mage 1, 20 and differs from MAGES 1-6. It is referred to as mage 7 hereafter. Additional screenings yielded MAGE 8-11. All MAGE sequences identified are presented as SEQ ID's.

Example 34

The usefulness of the TRAPs, as well as TRAs derived 25 therefrom, was exemplified by the following.

Exon 3 of MAGE 1 was shown to transfer expression of antigen E. As a result, it was decided to test whether synthetic peptides derived from this exon 3 could be used to confer sensitivity to anti-E CTL.

To do this, and using standard protocols, cells 30 normally insensitive to anti-E/CTLs were incubated with the synthetic peptides derived from Exon 3.1. Using the CTL lytic assays described supra on P815A, and a peptide concentration of 3 mM, the peptide Glu-Ala-Asp-Pro-Thr-Gly-His-Ser-Tyr (SEQ ID NO: 26) was shown to be best. The 35

assay showed lysis of 30%, indicating conferring of sensitivity to the anti-E CTL.

**Example 35**

5 Nucleic acid sequences referred to as "smage" were isolated from murine cells. Using the protocols described supra, a cosmid library was prepared from the DNA of normal DBA/2 kidney cells, using cosmid vector C2RB. As a probe, the 2.4 kb BamHI fragment of MAGE-1 was used. The DNA was blotted to nylon filters, and these were washed in 2xSSC at 10 65°C to identify the smage material.

**Example 36**

Further tissue samples were tested for the presence of MAGE genes, using the protocols discussed supra. Some of these results follow.

15 There was no expression of the MAGE genes in brain or kidney tumor tissue. Colon tumor tissue showed expression of MAGE 1, 2, 3 and 4, although not all tumors tested showed expression of all MAGE genes. This is also true for 20 pancreatic tumor (MAGE 1); non-small cell lung (MAGE 1, 2, 3 and 4), prostate (MAGE 1), sarcomas (MAGE 1, 2, 3 and 4), breast (MAGE 1, 2 and 3), and larynx (MAGE 1 and 4).

**Example 37**

25 A cytolytic CTL clone "20/38" was obtained from peripheral blood lymphocytes of melanoma patient MZ2. This clone is described by Van den Eynde et al., Int. J. Cancer 44: 634-640 (1989), the disclosure of which is incorporated by reference. The CTL clone was isolated following Herin et al., Int. J. Cancer 39: 390-396 (1987), which is incorporated by reference. The assay is described herein, 30 however. Autologous melanoma cells were grown in vitro, and then resuspended at  $10^7$  cells/ml in DMEM, supplemented with 10% HEPES and 30 mM FCS, and incubated for 45 minutes at 37°C with 200  $\mu$ Ci/ml of  $\text{Na}^{51}\text{CrO}_4$ . Labelled cells were washed three times with DMEM, supplemented with 10 mM

5 HEPES. These were then resuspended in DMEM supplemented with 10 mM HEPES and 10% FCS, after which 100  $\mu$ l aliquots containing  $10^3$  cells, were distributed into 96 well microplates. Samples of the CTL clone were added in 100  $\mu$ l of the same medium, and assays were carried out in duplicate. Plates were centrifuged for four minutes at 100g, and incubated for four hours at 37°C in a 5.5% CO<sub>2</sub> atmosphere.

10 Plates were centrifuged again, and 100  $\mu$ l aliquots of supernatant were collected and counted. Percentage of <sup>51</sup>Cr release was calculated as follows:

$$\% \text{ } ^{51}\text{Cr release} = \frac{(\text{ER-SR})}{(\text{MR-SR})} \times 100$$

15 where ER is observed, experimental <sup>51</sup>Cr release, SR is spontaneous release measured by incubating  $10^3$  labeled cells in 200  $\mu$ l of medium alone, and MR is maximum release, obtained by adding 100  $\mu$ l 0.3% Triton X-100 to target cells.

20 Those mononuclear blood samples which showed high CTL activity were expanded and cloned via limiting dilution, and were screened again, using the same methodology.

25 The same method was used to test target K562 cells. When EBV-B cells were used, the only change was the replacement of DMEM medium by Hank's medium, supplemented with 5% FCS.

These experiments led to isolation of CTL clone 20/38.

30 Figure 14 presents the results of these assays. Specifically, it will be seen that the CTL clone lysed autologous melanoma cell line MZ2-MEL.3.0, but did not lyse EBV-B cell lines, fibroblasts, K562 or non-autologous melanoma cell line SK-MEL-29.

Example 38

Once the CTL clone was recognized as being specific for the autologous cell line, it was tested for antigenic

specificity. To do this, antigen loss variants derived from melanoma cell line MEL-MZ2 were tested in the same type of chromium release assay described above. These target lines were MZ2-MEL 3.0, which is D<sup>+</sup>, E<sup>+</sup>, F<sup>+</sup>, A<sup>+</sup>, MZ2-5 MEL.61, which is D<sup>-</sup>, MZ2-MEL 2.2, which is E<sup>-</sup>, and MZ2-MEL.4, which is F<sup>-</sup>. In addition to CTL clone 20/38, clones which are known to be anti-A (CTL 28/336), anti-F (CTL 76/6), and anti-E (CTL 22/13) were tested.

10 These results are set forth in figure 15. It will be seen that CTL clone 20/38 lysed all the cell lines leading to chromium release except D<sup>-</sup> cell line MZ2-MEL.61, thus indicating that the CTL clone is anti-D. This result was confirmed, in experiments not included herein, by experiments where TNF release by the CTL clone was observed 15 only in the presence of melanoma lines presenting antigen D.

Example 39

20 Once antigen D was identified as the target molecule, studies were carried out to determine the HLA type which presented it. The experiments described in example 38 showed that antigen D was presented by MZ2-MEL, and this cell line's HLA specificity is known (i.e., A1, A29, B37, B44, Cw6, C.cl.10). It was also known, however, that a 25 variant of MZ2-MEL which had lost HLA molecules A29, B44 and C.cl.10 still expressed antigen D, so these could be eliminated from consideration. Studies were not carried out on lines expressing B37, as none could be found.

30 In all, 13 allogeneic lines were tested, which expressed either HLA-A1 (10 of 13), or Cw6 (3 of 13). The cell lines were tested for their ability to stimulate release of TNF by CTL clone 20/38, using the method of Traversari et al., Immunogenetics 35: 145-152 (1992), the disclosure of which is incorporated by reference. This assay measures TNF release via testing toxicity of 35 supernatants on WEHI 164-13 cells.

In the assays, cell samples (3000, 10,000 or 30,000 cells) from the allogeneic lines were cultured in the presence of 1500 cells of the CTL clone, and 25 u/ml of IL-2. Twenty-four hours later, the supernatant from the culture was tested against the WEHI cells for toxicity. The results are presented in Table 3, which follows.

Eight cell lines were found to stimulate TNF release from the CTL clone 20/38. All of these lines were HLA-A1. None of the Cw6 presenting lines did so.

The cell lines were also assayed to determine MAGE expression. All eight of the lines which stimulated TNF release expressed MAGE-3, whereas the two HLA-A1 lines which were negative did not.

Table 3

Melanoma	Number of Cells	TNF pg/ml		Expression of Mage-3		Expression of HLA- $\beta$ -1	
		Exp 1		Exp 2			
		+CTL 20/38	+CTL 20/38				
M22-MEL.61.2	50000	1	4	+++	+		
M22-MEL-ET1	50000 1666	>120 66	>120 >120	+++	+		
LY-1-MEL	30000 10000 3000	1 >120 <1	>120 >120 114	>120 >120 2	+++	+	
MI-10221	30000 10000 3000	<1 <1 <1	>120 71 74		+++	+	
LY-2-MEL	30000 10000 3000	1 >120 >120	57 86 91		+++	+	
LY-4-MEL	30000 10000 3000	1 >120 >120			+++	+	
SK23-MEL	30000 10000 3000	1 >120 >120	112 116 105		+++	+	
MI-665/2-MEL	30000 10000 3000	1 >120 >120	3 2 5,2	2 2 1	4 5 5	- + +	
LE34-MEL	30000 10000 3000	1 >120 >120			+++	+	
LB45-MEL	30000 10000 3000	1 >120 >120	11 6 2	1 1 <1	30 12 7	- + +	
NA-6-MEL	30000 10000 3000	1 >120 >120	77 104 110	5 5 4	98 >120 >120	+++ + +	
MI-13443-MEL	30000 10000 3000	1 >120 >120			+++	+	
LB5-MEL	30000 10000 3000	1 >120 >120	8 5 5	4 4 1	9 11 5	+- - -	
SK64-MEL	30000 10000 3000	1 >120 >120	4 2 1	2 1 1	5 5 4	?? - -	
LE33-MEL	30000 10000 3000			1 1 1	3,5 4 3	+++ - -	
LB73-MEL	50000		16		-	-	

1500 CTL 20/38 and 25 $\mu$ g/ml IL2 were mixed with the indicated number of cells of the different allogeneic melanomas. 24 hours later, the amount of TNF present in the supernatant was assayed by testing its cytotoxicity for WEHI-164-13 cells.

Example 40

In view of the results set forth in example 39, experiments were carried out to determine if antigen D was in fact a tumor rejection antigen derived from MAGE-3. To do this, recipient COS-7 cells were transfected with 100ng of the gene for HLA-A1 cloned into pcDNA I/Amp, and 100 ng of one of (a) cDNA for MAGE-1 cloned into pcDNA I/Amp, (b) cDNA for MAGE-2 cloned into pcDSR $\alpha$ , or (c) cDNA for MAGE-3 cloned into pcDSR $\alpha$ . The transfecting sequences were ligated into the plasmids in accordance with manufacturer's instructions. Samples of COS-7 cells were seeded, at 15,000 cells/well into tissue culture flat bottom microwells, in Dulbecco's modified Eagles Medium ("DMEM") supplemented with 10% fetal calf serum. The cells were incubated overnight at 37°C, medium was removed and then replaced by 30  $\mu$ l/well of DMEM medium containing 10% Nu serum, 400  $\mu$ g/ml DEAE-dextran, 100  $\mu$ M chloroquine, and the plasmids described above. Following four hours of incubation at 37°C, the medium was removed, and replaced by 50  $\mu$ l of PBS containing 10% DMSO. This medium was removed after two minutes and replaced by 200  $\mu$ l of DMEM supplemented with 10% of FCS.

Following this change in medium, COS cells were incubated for 24 hours at 37°C. Medium was then discarded, and 1500 cells of CTL clone 20/38 were added, in 100  $\mu$ l of Iscove's medium containing 10% pooled human serum, supplemented with 25 u/ml of IL-2. Supernatant was removed after 24 hours, and TNF content was determined in an assay on WEHI cells, as described by Traversari et al., Immunogenetics 35: 145-152 (1992), the disclosure of which is incorporated by reference. These results are shown in figure 16.

It will be seen that the CTL clone was strongly stimulated by COS-7 cells transfected with HLA-A1 and MAGE-3, but not by the cells transfected with the other Mage genes. This leads to the conclusion that antigen D is a tumor rejection antigen derived from the tumor rejection

antigen precursor coded by gene MAGE-3, and that this TRA is presented by HLA-A1 molecules.

Example 41

5 It is well known that different alleles of genes may produce different proteins. This principle should extend to the MAGE family of genes as well, and is an important consideration in view of diagnostic and therapeutic ramifications. Thus, polymorphism in the MAGE family was studied.

10 To address the issue of polymorphism, blood lymphocytes of ten individuals were collected, and genomic DNA extracted. This DNA was subjected to Southern blotting in accordance with James et al., Canc. Res. 48: 5546-5551 (1988), incorporated by reference. Briefly, the labelled 15 2.4 kb genomic DNA fragment of MAGE-1, containing the last two exons of MAGE-1, described supra, was hybridized with the filter carrying the digested DNA, at 42°C for at least 16 hours, in 50% formamide, 5% dextran sulfate, 6xSSC, 1% SDS and 0.1 mg/ml heterologous DNA. The hybridization 20 filters were washed, consecutively, in 2xSSC, 0.1% SDS (room temperature, 15 minutes), and twice in 0.1xSSC, 0.1% SDS at 67°C for 30 minutes, each wash. Autoradiography was carried out at -70°C for 7-10 days, using standard film.

25 A pattern of 13 hybridizing bands was observed, which was conserved over all individuals. One individual did show an additional band, but also showed the 13 band pattern.

Example 42

30 It was of interest to determine which chromosome or chromosomes bear the MAGE genes. To ascertain this, a panel of hamster/human somatic cell hybrids was used. The hybrids were obtained either from the Human Genetic Mutant Cell Repository ("GM" prefix), or from Johns Hopkins University ("A<sub>3</sub>" prefix). Each hybrid was cytogenetically 35 studied to determine human chromosome content.

Total genomic DNA of the hybrids was probed in the same manner described in Example 41, supra (the conditions of stringency used prevented cross hybridization with hamster DNA).

5 Table 4, which follows, summarizes the result of the probe work. Analysis of the data led to the conclusion that the pattern of hybridization was only concordant with location of MAGE-1 on the X chromosome.

TABLE 4 - Segregation of MAGE-1 with human chromosomes in human-hamster hybrid cell DNA

Hybrid	MAGE-1	Human chromosome		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y		
GM06317		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM06318B		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM07300		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM07301		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM08854		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM09142		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10095		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10115		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10156B		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10253		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10322		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10478		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10479		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10498		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10567		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10611		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10612		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10629		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10791		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10880		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GM10888		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A <sub>3</sub> ADA1D/2		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A <sub>3</sub> ADA6F5		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A <sub>3</sub> ADA13	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A <sub>3</sub> ADA14	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A <sub>3</sub> G1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A <sub>3</sub> HR20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
P <sub>8</sub> Me4	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Number of concordant hybrids	(++)	2	1	3	2	1	3	4	2	1	3	4	2	1	3	4	3	3	3	0	3	1	2	1	3	1	4
Number of discordant hybrids	(--)	17	19	14	14	15	18	18	16	17	18	17	17	16	14	18	17	19	18	16	14	15	18	17			
Percent discordancy		22	23	38	37	41	24	22	23	30	30	26	22	30	37	16	33	26	27	23	35	37	36	0	26		

+ = chromosome present; - = chromosome absent;

± = very faint bands indicating that only a small percentage of the cells contained the chromosome (not included in calculation of percent discordancy)

1,2 - GM09142 contains only part of chromosomes X and 21, der 21 1(X;21)

3,4 - GM10095 contains only part of chromosomes X and 9, der 9 1(X;9)

5 - PgMe4<sup>1</sup> contains a deleted chromosome 2 and is missing 2p23-p24

6 - A3G1 contains only the q arm of chromosome 6

Example 43

In this experiment, a study was carried out to determine if all twelve known MAGE genes were located on the X chromosome. This was accomplished via the use of polymerase 5 chain reaction ("PCR") technology.

RNA purification and cDNA synthesis were first carried out, in accordance with Weynants et al., Int. J. Cancer 56: 826-829 (1994), incorporated by reference herein. Next, 1/20 of the cDNA produced from 2 ug of total RNA was supplemented 10 with 5 ul of PCR buffer (500 mM KCl, 100 mM Tris pH 8.3), 1 ul each of 10 mM dNTPs, 25 pmoles of each primer (see below), 3 ul of 25 mM MgCl<sub>2</sub>, and 1.25 units of Taq polymerase, with water added to final volume of 50 ul.

The primers were as follows:

15 MAGE-3: 5'-TGGAGGACCAGAGGCC, 5'-GGACGATTATCAGGAGGCCTGC (725 bp) (SEQ ID NOS: 27 AND 28)  
MAGE-4: 5'-GAGCAGACAGGCCAACCG, 5'-AAGGACTCTGCGTCAGGC (446 bp) (SEQ ID NOS: 29 AND 30)  
MAGE-5: 5'CTAGAGGAGCACCAAAGGAGAAG, 5'-TGCTCGGAACACAGACTCTGG 20 (413 bp) (SEQ ID NOS: 31 AND 32)  
MAGE-6: 5'-TGGAGGACCAGAGGCC, 5'-CAGGATGATTATCAGGAAGCCTGT (727 bp) (SEQ ID NOS: 33 AND 34)  
MAGE-7: 5'-CAGAGGAGCACCGAACGGAGAA, 5'-CAGGTGAGCGGGTGTGTC (405 bp) (SEQ ID NOS: 35 AND 36)  
25 MAGE-8: 5'-CCCCAGAGAACCACTGAAGAAG, 5'-GGTGAGCTGGTCCGGG (399 bp) (SEQ ID NOS: 37 AND 38)  
MAGE-9: 5'-CCCCAGAGCAGCACTGACG, 5'-CAGCTGAGCTGGTCAACC (391 bp) (SEQ ID NOS: 39 AND 40)  
MAGE-10: 5'-CACAGAGCAGCACTGAAGGAG, 5'-CTGGTAAAGACTCACTGTCTGG 30 (485 bp) (SEQ ID NOS: 41 AND 42)  
MAGE-11: 5'-GAGAACCCAGAGGATCACTGGA, 5'-GGGAAAAGGACTCAGGGTCTATC (422 bp) (SEQ ID NOS: 43 AND 44)  
MAGE-12: 5'-GGTGGAAAGTGGTCCGCATCG, 5'-GCCCTCCACTGATCTTAGCAA (392 bp) (SEQ ID NOS: 45 AND 46)  
35 Amplification was carried out for 30 cycles (MAGE-3, 4, 6, 12) or 32 cycles (MAGE-5, 7-11), where a cycle was one minute at 94°C followed by two minutes at 65°C for MAGE-5, 7-12, or two

minutes at 68°C (MAGE-4), or two minutes at 71°C (MAGE-3 and MAGE-6); followed by three minutes at 72°C (MAGE-3, 5-12), or two minutes at 72°C (MAGE-4). The analysis was carried out on hybrid cell line GM 10868, which contains human chromosome 12, 5 and GM 07301, which contains chromosome 12 and the X-chromosome. All assays were negative with the human GM 10868 line, and all were positive with the GM 07301 cell line, which indicated that all 12 genes are found on the X-chromosome.

Example 44

10 The sizes of mRNAs for the different MAGE genes are similar, and thus Northern blot analysis cannot be used to determine expression of the various MAGE genes in different tissues, both normal and tumor. PCR analysis, along the lines of the study in example 43, supra, however, was believed to be 15 useful.

To this end, a series of various tumors and normal tissues were tested for expression of MAGE genes.

20 Total RNA of the cells tested was extracted, and was then oligo dT primed, following art known techniques. The resulting material was then subjected to PCR, following the protocols of example 43, supra. For MAGE-1 and MAGE-2, the protocols of Brasseur et al., Int. J. Cancer. 52: 839-841 (1992), and DeSmet et al., Immunogenetics 39: 121-120 (1994), both of which are incorporated by reference, were used.

25 Table 5, which follows, elaborates these results, with a representative but by no means exhaustive listing of tissues tested. Each of MAGE 1-4, 6 and 12 showed significant expression in a number of tumors of varied tissue types. MAGE-5 and 8-11 were expressed very weakly in all tissues tested, whereas MAGE-7 RNA was not detectable at all. With respect to normal tissues, including tissues taken from a >20 week fetus, all were negative for MAGE RNA but for testis and placenta. Testis expressed all MAGE genes but MAGE-7, while placenta expressed MAGE-3, 4, and 8-11.

TABLE 5. Expression of Mage-1, 2, 3, 4, 6 and -12 by tumors and normal tissues

	MAGE 1	MAGE 2	MAGE 3	MAGE 4	MAGE 6	MAGE 12
<b>COLON CARCINOMAS</b>						
MZ-CO-2 1	++	++	+	-	-	+
SK-CO-11 1	-	++	+++	-	+	++
LB150 22	-	-	-	+	-	-
HSR 320 1	-	+++	+++	+	++	+++
<b>LEUKEMIAS</b>						
K562 1	-	++	+++	-	++	+++
<b>MELANOMAS</b>						
MI10221 1	-	+++	+++	+++	+++	+++
MZ2-MEL 3.0 1	+++	+++	+++	-	+++	+
LB265 22	-	++	-	-	-	+
LG7 22	-	++	-	-	-	-
LG11 22	++	++	++	-	-	+++
LB271 22	-	++	+++	-	++	+++
<b>LUNG CANCERS</b>						
LB178 (NSCLC) 22	++	-	-	+++	-	-
LB175 (NSCLC) 22	-	++	+++	+++	-	+++
LB11 (SCLC) 1	++	+++	+++	-	-	+++
LB12 (SCLC) 1	-	+++	+++	-	-	+++
<b>SARCOMAS</b>						
LB23 1	-	-	-	++	-	-
LB408 22	-	-	-	++	-	-
LB258 22	+	++	+	-	-	++
<b>BREAST CARCINOMAS</b>						
LB280 22	++	-	++	-	-	+
LB284 22	++	++	++	+	-	++
<b>Normal Tissues</b>						
Stomach	-	-	-	-	-	-
Lung	-	-	-	-	-	-
Breast	-	-	-	-	-	-
Colon	-	-	-	-	-	-
Skin	-	-	-	-	-	-
Uterus	-	-	-	-	-	-
Testis	++	++	++	++	++	++
Thymocytes	-	-	-	-	-	-
EBV-lymphocytes	-	-	-	-	-	-
Foetal liver	-	-	-	-	-	-
Foetal brain	-	-	-	-	-	-
Placenta LB694	-	-	+	+++	-	-

RNA from tumor cell lines (1), tumor samples (22) and normal tissues were tested by RT-PCR for the expression of MAGE genes. PCR primers were chosen as indicated in methods. For MAGE-12, PCR amplification of RNA in the absence of reverse transcription indicated that in our conditions the contamination by genomic DNA was negligible. The level of expression evaluated by band intensity of PCR products fractionated in agarose gels is represented by +++, ++, +. Absence of product is indicated by -.

Example 45

The expression of the MAGE-1, 2 and 3 genes in various tumors and normal tissues was evaluated, using both reverse transcription and polymerase chain reaction ("PCR") amplification. To perform these assays, the total RNA of the cells of interest was extracted via the well known guanidine-isothiocyanate procedure of Davis et al., Basic Methods in Molecular Biology, 1986 (New York, Elsevier, pp. 130), which is incorporated by reference in its entirety. cDNA was then synthesized, by taking 2 ug of the RNA, diluting it with water, and then adding the following materials: 4 ul of 5X reverse transcriptase buffer, 1 ul each of each dNTP (10 mM), 1 ul of a 40  $\mu$ M solution of oligo dT(15), 20 units of RNAsin, 2 ul of 0.1 M dithiothreitol, and 200 units of MoMLV reverse transcriptase. All materials were mixed in a 20 ul reaction volume, and incubated at 42°C for 60 minutes and diluted to 100 ul with water.

Presence or absence of each of MAGE-1, -2, and -3 cDNA was detected via PCR amplification, in separate reactions, using oligonucleotide primers located in different exons of the MAGE gene of interest. For MAGE-1, the primers were:

5'-CGGCCGAAGAACCTGACCCAG-3'  
(SEQ ID NO: 47)

25 5'-GCTGGAACCCCTCACTGGGTTGCC-3'  
(SEQ ID NO: 48)

These are described by Brasseur et al., Int. J. Cancer 52: 839-841 (1992).

For MAGE-2, the primers were:

30 5'-AAGTAGGACCCGAGGCCTG-3'  
(SEQ ID NO: 49)

5'-GAAGAGGAAGAACGGTCTG-3'  
(SEQ ID NO: 50)

(DeSmet et al., Immunogenetics 39: 121-129 (1994)).

For MAGE-3, the primers were:

5'-TGGAGGACCCAGAGGCC-3'  
(SEQ ID NO: 27)

5

5'-GGACGATTATCAGGAGGCCTGC-3'  
(SEQ ID NO: 28)

(Serial No. 08/204,727 filed March 1, 1994 to Gaugler et al. incorporated by reference).

For each PCR reaction, 5 ul of cDNA were supplemented with 5 ul of 10XPCR buffer, 1 ul of each dNTP (10 mM), 1 ul each of 40  $\mu$ M primer solutions, 1.25 units of Taq polymerase, and water, to a total volume of 50 ul. Each mixture was heated for five minutes at 94°C. Amplification was then carried out for 30 cycles (MAGE-1: 1 minute at 94°C, 3 minutes at 72°C; MAGE-2: 1 minute at 94°C, 2 minutes at 67°C; MAGE-3: 1 minute at 94°C, 4 minutes at 72°C). Cycling was concluded, in each case, with a final extension at 72°C for 15 minutes. A 10 ul sample of each reaction was run on a 1% agarose gel, and visualized by ethidium bromide fluorescence. To ensure that RNA was not degraded, a PCR assay with primers specific for  $\beta$ -actin was carried out, following the listed protocols, except that only 20 cycles were carried out with the annealing step at 65°C. Data are summarized in the Table which follows:

Table 6 Expression of gene MAGE-1, 2 and 3 in lung tumors

	Proportion of positive samples		
	MAGE-1	MAGE-2	MAGE-3
<b>Non-small cell lung cancer</b>	<b>16/46</b>	<b>16/46</b>	<b>14/46</b>
squamous cell carcinoma	8/26	6/26	7/26
adenocarcinoma	8/18	9/18	7/18
large cell carcinoma	0/2	1/2	0/2
<b>Small cell cancer</b>	<b>1/3</b>	<b>2/3</b>	<b>2/3</b>
<b>Normal lung samples</b>	<b>0/8</b>	<b>0/8</b>	<b>0/8</b>

Example 46

The previous example showed how to identify expression of various MAGE genes. This example explains quantitation of the expression.

First, cDNA was synthesized in the same way described in example 1, except that the oligo dT consisted solely of dT15, and the reaction mixture was preincubated at room temperature for 10 minutes to optimize annealing. Also, following the incubation, the transcriptase activity was terminated by heating the mixture at 95°C for 15 minutes. PCR amplification was carried out, by combining 5 ul of 10x PCR buffer, 0.5 ul of a 2.5 mM dNTP mix, 0.2 µCi of  $\alpha^{32}\text{P}$ -dCTP, 0.5 ul of each primer (40 µM solution), 1.25 units of Taq polymerase, and water, to a total of 50 ul. The mixtures were chilled on ice, and then 5 ul of chilled cDNA solution (100 ng total RNA) were added thereto. The

5 mixture was heated to 94°C for five minutes, and 24 cycles  
of amplification were carried out (one minute at 94°C,  
three minutes at 72°C per cycle). Cycling concluded with  
a final extension at 72°C, for 15 minutes. A 15 ul sample  
10 of PCR product was run on an agarose gel which was then  
fixed in 10% trichloroacetic acid for 30 minutes, dried,  
and then exposed to a phospho-screen for 90 minutes before  
scanning by Phosphor-Imager to measure incorporated  $^{32}\text{P}$ .  
This was compared to the incorporations from various  
15 dilutions of RNA of reference melanoma cell line MZ2-MEL-  
3.0.

15 Quantitative measurements of  $\beta$ -actin messenger and  
"GAPDH" (i.e., glyceraldehyde 3-phosphate dehydrogenase)  
was carried out on each cDNA sample, under similar  
conditions. The one difference was that only 18  
amplification cycles were carried out. A separate PCR  
reaction was set up with primers for  $\beta$ -actin and GAPDH,  
with only  $\beta$ -actin used for normalization. Results were  
expressed via formula:

20

$$\frac{100 \times \frac{\text{MAGE-1-S}}{\text{Actin-S}}}{\frac{\text{MAGE-1-MEL}}{\text{Actin-MEL}}}$$

25

where: S = product from tumor sample  
MEL = product from MZ2-MEL 3.0

30 The results obtained were comparable to those obtained  
previously with melanoma tumors. Level of expression  
varied, from 1 to 160% of the amount expressed by the  
reference cell line. Figure 2 presents some of these  
results (i.e., normalized results, relative to levels of  $\beta$ -  
actin expression). Values are percent of the level of  
35 MAGE-1 expression measured with RNA of the reference line

MZ2-MEL-3.0. Values are for MAGE-1 positive tumors of Table 2). Table 7, which follows, summarizes patterns of expression for various tumors.

Table 7. Pattern of expression of genes MAGE-1,2 and 3 by MAGE-positive lung tumor samples

	MAGE-1°	MAGE-2	MAGE-3
<b>Squamous cell carcinoma</b>			
LB 175	++	++	+++
LB 178	++	-	-
LB 182 (A1)*	-	+	-
LB 195	+	++	+++
LB 206	+++	+	++
LB 321	+	-	-
LB 323	+++	+	+++
LB 424	+	-	+
LB 425	-	-	+
LB 498 (A1)	+++	-	-
LB 557	-	+++	+++
<b>Adenocarcinoma</b>			
LB 117 (A1)	+	++	++
LB 212	++	+	-
LB 264 (A1)	+++	++	+++
LB 292	-	++	+++
LB 306	++	+	++
LB 322	-	+	+
LB 474 (A1)	+	++	-
LB 497	++	+++	+++
LB 510	+++	-	-
LB 558 (A1)	+	+	+
<b>Large cell carcinoma</b>			
LB 259	-	+	-
<b>Small cell lung cancer</b>			
LB 444	-	++	+++
LB 648 (A1)	+	++	+++

Example 47

The expression of the MAGE-3 gene in various tumors and normal tissues was evaluated, using both reverse transcription and polymerase chain reaction ("PCR") amplification. To perform these assays, the total RNA of the cells of interest was extracted via the well known guanidine-isothiocyanate procedure of Davis et al., Basic Methods in Molecular Biology, 1986 (New York, Elsevier, pp. 130), which is incorporated by reference in its entirety. cDNA was then synthesized, by taking 2 ug of the RNA, diluting it with water, and then adding the following materials: 4 ul of 5X reverse transcriptase buffer, 1 ul each of each dNTP (10 mM), 2 ul of a 20  $\mu$ M solution of oligo dT, 20 units of RNAsin, 2 ul of 0.1 M dithiothreitol, and 200 units of MoMLV reverse transcriptase. All materials were mixed in a 20 ul reaction volume, and incubated at 42°C for 60 minutes. For the amplification reaction, 1/20 of the cDNA reaction product was supplemented with 5 ul of PCR buffer, 0.5 ul of each of the dNTPs (10 mM), 1 ul each of 20  $\mu$ M solutions of primer (see infra), and 1.25 units of Taq polymerase. Water was added to a final volume of 50 uls. The primers used for MAGE-3 were:

25 5'-TGGAGGACCAGAGGCC-3'  
(SEQ ID NO: 27)

5'-GGACGATTATCAGGAGGCCTGC-3'  
(SEQ ID NO: 28)

These correspond to a sense sequence in exon 2 of the gene (SEQ ID NO: 27), and an antisense sequence in exon 3 (SEQ 30 ID NO: 28).

PCR was performed for 30 cycles (one minute at 94°C, four minutes at 72°C). PCR products were size fractionated on a 1% agarose gel, and then analyzed. The results are presented in the table which follows. These data confirm

some results obtained previously, but also show the expression of MAGE-3 in head and neck squamous cell carcinomas, a result not suggested by previous work.

Table 8. Expression of gene MAGE-3 by tumoral, normal and fetal tissues.

TUMORS	NORMAL TISSUES		
	HISTOLOGICAL TYPE		MAGE-3 expression*
	Number of cell lines	Number of MAGE-3 positive tumors*	
Melanomas	50/62 (81%)	72/105 (69%)	-
Head and neck squamous cell carcinomas	-	20/36 (56%)	-
Lung carcinomas	1/2	14/46 (30%)	-
NSCLC ‡	18/22 (82%)	2/3	-
SCLC	5/16	5/31 (16%)	++
Colorectal carcinomas	2/6	16/132 (12%)	-
Mammary carcinomas	-	2/6	-
Bladder tumors	-	2/6	-
Sarcomas	1/4	3/10	-
Prostatic carcinomas	-	3/20	-
Renal carcinomas	0/5	0/18	-
Leukemias	2/6	0/20	-
Lymphomas	0/6	0/5	-

\*Expression of gene MAGE-3 was tested by RT-PCR amplification on total RNA, with the primers described in methods. These primers distinguish MAGE-3 from the 11 other MAGE genes that have been identified.  
‡ NSCLC are non-small cell lung carcinomas, SCLC are small cell lung carcinomas.

Example 48

Bladder tumor specimens were collected at surgery. They were divided into two portions, one of which was used for routine histopathological evaluation. The other portion was frozen in liquid nitrogen immediately after transurethral resection, or radical cystectomy. These frozen samples were stored at -80°C until used for RNA extraction. Normal bladder tissue was obtained by biopsies of cadavers from donors in an organ transplant program.

Total RNA was extracted from the samples by the classic guanidine-isothiocyanate/cesium chloride method of Davis et al, Basic Methods in Molecular Biology, pp. 130-135, Elsevier, New York (1986). Synthesis of cDNA was then carried out by extension with oligo(dT) using 2 ug of RNA in a 20 ul reaction volume following DeSmet et al., Immunogenetics 39: 121-129 (1994), incorporated by reference herein. Following incubation at 42°C for one hour, the cDNA reaction mixture was diluted to 100 ul with water. Separate polymerase chain reaction amplification were then carried out to determine whether any of MAGE-1, 2, 3 or 4 cDNA were present. The amplifications were carried out using oligonucleotide primers located in different exons of the MAGE genes. PCR amplification was also carried out using primers for HLA-A1.

The primers used were the following:

5'-TGGAGGACCAGAGGCC-3' (sense, exon 2) (SEQ ID NO: 27) and

5'-GGACGATTATCAGGAGGCC-3' (antisense, exon 3) (SEQ ID NO: 28) for MAGE-3

5'-CGGCCGAAGAACCTGACCCAG-3' (sense, exon 1) (SEQ ID NO: 47) and

5'-GCTGGAACCTCACTGGGTTGCC-3' (anti-sense, exon 3) (SEQ ID NO: 48) for MAGE-1

5'-AAGTAGGACCCGAGGCAG-3' (sense, exon 2) (SEQ ID NO: 49) and

5'-GAAGAGGAAGAACCGGGTCTG-3' (anti-sense, exon 3) (SEQ ID NO: 50) for MAGE-2

5'-GAGCAGACAGGCCAACCG-3' (sense, exon 2) (SEQ ID NO: 29)  
and

5'-AAGGACTCTGCGTCAGGC-3' (anti-sense, exon 3) (SEQ ID NO: 30) for MAGE-4

5 5'-GGGACCAGGAGACACGGAATA-3' (sense, exon 2) (SEQ ID NO: 51)  
and

5'-AGCCCGTCCACGCACCG-3' (anti-sense, exon 3) (SEQ ID NO: 52) for HLA-A1

10 SEQ ID NOS: 27 and 28 are described by Weynants et al.,  
Int. J. Cancer 56: 826-829 (1994). SEQ ID NOS: 47 and 48  
are described in Brasseur et al., Int. J. Cancer 52: 839-  
841 (1992). SEQ ID NOS: 49 and 50 are disclosed in DeSmet  
et al., Immunogenetics 39: 121-129 (1994). SEQ ID NOS: 29  
and 30 are disclosed in copending application Serial No.  
15 08/299,849 filed September 1, 1994 to DePlaen et al., and  
incorporated by reference. SEQ ID NOS: 51 and 52 are found  
in Gaugler et al., J. Exp. Med. 179: 921-930 (1994), as  
well as the above-identified parent application. All of  
these references are incorporated by reference.

20 The amplification protocol was as follows. Each PCR  
reaction used 5 ul of cDNA, supplemented with 5 ul of 10x  
PCR buffer, 1 ul each of 10 mM dNTP, 0.5 ul each of 80 uM  
solutions of primers, 1.25 units of Taq DNA polymerase, and  
water to achieve a total volume of 50 ul. The mixtures  
25 were heated to 94°C for 5 minutes, followed by  
amplification in a thermal cycler, for 30 cycles. For  
MAGE-1, 1 cycle was one minute at 94°C followed by three  
minutes at 72°C. For MAGE-2, one cycle was 94°C for one  
minute, followed by two minutes at 67°C and two minutes at  
30 72°C. For MAGE-3, one cycle was one minute at 94°C;  
followed by four minutes at 72°C. For MAGE-4, one cycle  
was one minute at 94°C, two minutes at 68°C, and two  
minutes at 72°C. The cycle for HLA-A1 was the same as that  
for MAGE-4. A 10 ul sample of each reaction was run on a  
35 1% agarose gel, and then visualized by ethidium bromide  
fluorescence. In order to provide a control for RNA

integrity, a 20 cycle PCR assay, using primers specific for  $\beta$  actin, was carried out in each case, following Weynants et al., supra.

5 The protocols described were developed with certain goals in mind. Primers were selected so as to be in different exons, thus preventing false positives due to DNA contamination of the RNA preparations. Under the conditions used, DNA generates either no PCR product, or longer products which are readily distinguishable from 10 amplified cDNA. This is shown by figure 19. In figure 19, a bladder tumor sample from a patient, referred to as "HM15" is shown in each "R" lane. Lanes marked "D" show 15 products obtained from amplification of the patients' genomic DNA. The PCR products were run on a 2.5% low melting agarose gel, but the assays were identical to the protocol of this example in all other ways. Size markers are on the left hand side. There was no band in the MAGE-1 reaction, because of the large intron between the two primers.

20 Table 10, which follows, shows the results obtained for a number of tumors (nomenclature is explained below). Of 57 samples of primary transitional cell carcinoma, 21% expressed MAGE-1, 30% expressed MAGE-2, 35% expressed MAGE-3, and 33% expressed MAGE-4. Ta tumors and low grade T1 25 tumors expressed none of these, or expressed only a single gene, at low levels. Higher stage tumors, in contrast, frequently expressed high levels of several genes. It was also found that the fraction of invasive tumors which expressed MAGE genes was 2-5 times higher than the fraction 30 observed with superficial tumors, as is depicted in figure 2 (this figure is based upon data from Table 10). Tumors expressing at least one of the four MAGE genes accounted for 61% of the 28 invasive tumors studied. Among the 29 superficial tumors, the proportion was only 28%. Results 35 paralleled other results reported previously for melanoma, in that all but one of the tumors expressing MAGE-1 also expressed MAGE-3.

None of the six biopsies of normal bladder examined expressed any of the MAGE genes discussed herein.

In some instances, several tumor samples were obtained from the same patient. The analysis of these patients is set out in Table 9. Patient HM61 had a primary tumor and an invaded lymph node. They displayed a very similar pattern of expression of MAGE-1, 2, and 3, with MAGE-1 predominating. Normal mucosa adjacent to the tumor was completely negative for MAGE-2 and MAGE-3, with a very low level of MAGE-1 expression, which was probably due to the presence of a few tumor cells. In patient "HM25", the initial tumor, and an early recurrence, both expressed MAGE-1, 2, 3 and 4. A recurrence which occurred two years after the first displayed a very different pattern, expressing only MAGE-2 and MAGE-3. A similar discordance between primary tumor and recurrence was observed with patient "HM20". Patients HM30 and LB526 showed differences in the pattern of MAGE-expression in different samples of the same primary tumor.

In the tables which follow, "Ta" stands for a superficial lesion, limited to bladder mucosa (also known as "stage Ta"). "Stage T1", or "T1" is used for superficial lesions limited to subepithelial connective tissue. "Stages T2-T4", or "T2-T4" refer to tumors which have invaded bladder muscle. The nomenclature "G1", "G2" and "G3" refers to the degree of differentiation, or histopathological grade. "G1" superficial tumor is well differentiated, while a "G3" tumor is poorly differentiated. See Mostofi et al., "Histological Typing of Urinary Bladder Tumors. WHO International Histological Classification of Tumors" (1973).

TABLE 9 - EXPRESSION OF GENES MAGE-1, 2, 3 AND 4  
IN MULTIPLE SAMPLES FROM BLADDER CARCINOMA PATIENTS

Patients	Samples	Tumor stage	and grade	MAGE-1	MAGE-2	MAGE-3	MAGE-4
HM 61	Primary tumor			-	-	-	-
	Metastatic iliac lymph node			++	+	+	+
	Mucosa adjacent to the tumor		+	-	-	-	-
HM 25	Primary tumor	T2 G2	+	++	+	++	++
	Tumor recurrence after 1 month	T2 G2	++	++	++	++	++
	Tumor recurrence after 2 years	T1 G2	-	+++	+++	-	-
HM 20	Primary tumor	T1 G1	+	-	-	-	-
	Tumor recurrence after 2 months	T1 G1	-	-	-	-	-
HM 30	Primary tumor, 1st sample	T2 G2	-	-	+	-	-
	Primary tumor, 2nd sample	T2 G2	-	-	-	-	-
LB 526	Primary tumor, radical cystectomy	T3 G2	+	+	++	+	+
	Primary tumor, 9-day pre-operative biopsy	T3 G2	+	+	+	-	-

Table 10. EXPRESSION OF GENES MAGE-1, 2, 3 AND 4 IN BLADDER TRANSITIONAL-CELL CARCINOMA SAMPLES

Tumor Stage and Grade <sup>‡</sup>		Patients	MAGE-1	MAGE-2	MAGE-3	MAGE-4 <sup>§</sup>
<b>Superficial tumors (n=29)</b>						
T <sub>a</sub> (n=7)	G1	HM 7 HM 32 (A1) HM 33 (A1) HM 49	- - - -	- - - -	+	-
	G2	LB 523 LB 817 LB 818	- - -	- - -	- - -	-
T <sub>1</sub> (n=22)	G1	HM 2 HM 6 (A1) HM 17 HM 20 HM 22 HM 34 HM 35	- - - + - - -	- - - - - - -	- - - - - - -	-
	G2	HM 4 HM 5 HM 9 HM 27 HM 37 HM 38 (A1) HM 39 HM 40 (A1) HM 41	- - - - - - - - -	- + - - - - - - -	- + - - - - - - -	- - - - +++ - - - -
	G3	HM 14 HM 23 HM 26 HM 42 (A1) HM 53 LB 767 (A1)	++ - - - - -	+++ - - - - -	+++ - - - - -	++ - - - - -
<b>Invasive tumors (n=28)</b>						
T <sub>2</sub> (n=15)	G2	HM 8 HM 13 (A1) HM 24 (A1) HM 25 HM 30 LB 796	- - + + - -	- - +++ ++ - -	- - +++ + - -	- - ++ ++ - -
	G3	HM 3 (A1) HM 10 HM 12 HM 15 HM 61 (A1) LB 524 (A1) LB 824 LB 825 LB 831	- - - +++ ++ - - - -	- - + - +++ + - - -	- - - +++ +++ - - - -	- - - - +++ - - - -
T <sub>3</sub> (n=11)	G2	HM 44 HM 45 HM 46 (A1) LB 526	- - - +	- - +++ -	- - +++ -	- - - +
	G3	HM 11 HM 18 HM 21 HM 47 HM 48 HM 50 HM 52 (A1)	++ - - +++ +++ - -	+	++ - - +++ +++ - -	++ - - - - - -
T <sub>4</sub> (n=2)	G3	HM 1 HM 51	- -	- -	- -	+

The foregoing examples show that expression of MAGE tumor rejection antigen precursors is correlated to various cancers. One aspect of the invention, then, is a method for determining these cancers by assaying a sample for expression of at least one MAGE tumor rejection antigen precursor. As MAGE genes are nearly without exception expressed only by tumor cells, there can be no question but that expression of a MAGE gene or genes is indicative of cancer. The fact that the cancer is a particular type, such as lung adenocarcinoma, is easily ascertainable, as adenocarcinoma cells have distinct morphologies which are identifiable by the skilled artisan. Similarly, the fact that the tumor of interest is a lung adenocarcinoma as compared to a tumor from a different body part is self evident; one does not find lung adenocarcinoma in, e.g., large intestine tissue. Analogous statements can be made for bladder and other cancers.

The assay for the MAGE genes can take many forms. Most preferably, the assay is done via determining gene expression, such as by determining mRNA transcription products. For example, amplification protocols, including but not being limited to polymerase chain reaction (PCR), and ligase chain reaction (LCR), are preferred. The assay can also be carried out using nucleic acid molecule probes, which are labelled or unlabelled, and which specifically hybridize to sequences characteristic of the MAGE gene of interest. Labelling nucleotide probes is well known to the art, labels including radioactive, fluorescent, chromophoric, magnetic, and other identifiable materials. Antibodies, haptens such as biotin, (strept)avidin, digoxin, digoxigenin, and so forth, can all be used. Non-labelled probes can also be used. In such a case, the probes will form a double stranded molecule with their target. Any remaining single stranded material can be enzymatically digested, and when something remains, it is a sign of MAGE expression. For the case of polymerase chain reaction or other methodologies where a primer or

primers are required, the molecules represented by SEQ ID NO: 47 and SEQ ID NO: 48 are especially preferred for MAGE-1, SEQ ID NO: 49 and 50 for MAGE-2, SEQ ID NOS: 27 and 28 for MAGE-3 and SEQ ID NOS: 29 and 30 for MAGE-4. 5 Similarly, these molecules are preferred as probes.

Quantitation of MAGE expression is shown herein as well. This is an important feature of the invention because in a given tumor sample (as compared to tumor cell lines) there will always be an undetermined proportion of 10 normal cells.

One may also assay for the expression product of the MAGE gene, e.g., the tumor rejection antigen precursor protein, via assays such as immunoassays. See, e.g., U.S. Patent Application Serial No. 08/190,411 filed February 1, 15 1994, and Chen, et al., Proc. Natl. Acad. Sci. USA 91(3): 1004-1008 (1994), both of which are incorporated by reference, teaching MAGE-1 specific mAbs.

The terms and expressions which have been employed are used as terms of description and not of limitation, and 20 there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

## (1) GENERAL INFORMATION:

(i) APPLICANTS: De Plaen, Etienn ; Boon-Falleur, Thierry; Lethé, Bernard; Szikora, Jean-Pierre; De Smet, Charles; Chomez, Patrick; Weynants, P.; Brasseur, Francis; Marchand, M.; Gaugler, Béatrice; Van den Eynde, Benoit; van der Bruggen, Pierre; Patard, Jean-Jacques

(ii) TITLE OF INVENTION: Method For Determining A Cancerous Condition by Assaying For Expression Of One Or More Mage Tumor Rejection Antigen Precursors

(iii) NUMBER OF SEQUENCES: 56

(iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: Felfe & Lynch  
(B) STREET: 805 Third Avenue  
(C) CITY: New York City  
(D) STATE: New York  
(F) ZIP: 10022

(v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Diskette, 5.25 inch, 360 kb storage  
(B) COMPUTER: IBM  
(C) OPERATING SYSTEM: PC-DOS  
(D) SOFTWARE: Wordperfect

(vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: Hanson, Norman D.  
(B) REGISTRATION NUMBER: 30,946  
(C) REFERENCE/DOCKET NUMBER: LUD 5356-PCT

(ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: (212) 688-9200  
(B) TELEFAX: (212) 838-3884

## (2) INFORMATION FOR SEQUENCE ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 462 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ACCACAGGGAG	AATGAAAAAGA	ACCCGGGACT	CCCAAAGACG	CTAGATGTGT	50
GAAGATCCCTG	ATCACTCATT	GGGTGTCTGA	GTTCTGCGAT	ATTCATCCCT	100
CAGCCAATGA	GCTTACTGTT	CTCGTGGGGG	GTTTGTGAGC	CTTGGGTAGG	150
AAAGTTTGCA	AGTCCCGCCT	ACAGCTCTAG	CTTGTGAATT	TGTACCCCTT	200
CACGTAAAAA	AGTAGTCCAG	AGTTTACTAC	ACCCCTCCCTC	CCCCCTCCCA	250
CCTCGTGTG	TGCTGAAGTTT	AGAAGTCTTC	CTTATAGAAG	TCTTCCGTAT	300
AGAACTCTTC	CGGAGGAAGG	AGGGAGGACC	CCCCCCCTTT	GCTCTCCAG	350
CATGCATTGT	GTCAACGCCA	TTGCACTGAG	CTGGTCGAAG	AAGTAAGCCG	400
CTAGCTTGCG	ACTCTACTCT	TATCTTAACT	TAGCTCGGCT	TCCTGCTGGT	450
ACCCCTTTGTG	CC				462

## (2) INFORMATION FOR SEQUENCE ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 675 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA GGT GGT	48
Met Ser Asp Asn Lys Lys Pro Asp Lys Ala His Ser Gly Ser Gly Gly	
5 10 15	
GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG TAC TCC CTG GAA	96
Asp Gly Asp Gly Asn Arg Cys Asn Leu Leu His Arg Tyr Ser Leu Glu	
20 25 30	
GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC TTC GCT GTT GTC ACA ACA	144
Glu Ile Leu Pro Tyr Leu Gly Trp Leu Val Phe Ala Val Val Thr Thr	
35 40 45	
AGT TTT CTG GCG CTC CAG ATG TTC ATA GAC GCC CTT TAT GAG GAG CAG	192
Ser Phe Leu Ala Leu Gln Met Phe Ile Asp Ala Leu Tyr Glu Glu Gln	
50 55 60	
TAT GAA AGG GAT GTG GCC TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC	240
Tyr Glu Arg Asp Val Ala Trp Ile Ala Arg Gln Ser Lys Arg Met Ser	
65 70 75 80	
TCT GTC GAT GAG GAT GAA GAC GAT GAG GAT GAT GAC TAC TAC	288
Ser Val Asp Glu Asp Asp Glu Asp Asp Glu Asp Asp Tyr Tyr	
85 90 95	
GAC GAC GAG GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT	336
Asp Asp Glu Asp Asp Asp Asp Ala Phe Tyr Asp Asp Glu Asp Asp	
100 105 110	
GAG GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA GAT GAG	384
Glu Glu Glu Leu Glu Asn Leu Met Asp Asp Glu Ser Glu Asp Glu	
115 120 125	
GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA GCT GAG GAA ATG	432
Ala Glu Glu Glu Met Ser Val Glu Met Gly Ala Gly Ala Glu Glu Met	
130 135 140	
GGT GCT GGC GCT AAC TGT GCC TGT CCT GGC CAT CAT TTA AGG AAG	480
Gly Ala Gly Ala Asn Cys Ala Cys Val Pro Gly His His Leu Arg Lys	
145 150 155 160	
AAT GAA GTG AAG TGT AGG ATG ATT TAT TTC TTC CAC GAC CCT AAT TTC	528
Asn Glu Val Lys Cys Arg Met Ile Tyr Phe Phe His Asp Pro Asn Phe	
165 170 175	
CTG GTG TCT ATA CCA GTG AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT	576
Leu Val Ser Ile Pro Val Asn Pro Lys Glu Gln Met Glu Cys Arg Cys	
180 185 190	

GAA AAT GCT GAT GAA GAG GTT GCA ATG GAA GAG GAA GAA GAA GAG	624
Glu Asn Ala Asp Glu Glu Val Ala Met Glu Glu Glu Glu Glu Glu	
195 200 210	
GAG GAG GAG GAG GAA GAG GAA ATG CGA AAC CCG GAT GGC TTC TCA CCT	672
Glu Glu Glu Glu Glu Glu Met Gly Asn Pro Asp Gly Phe Ser Pro	
220 225 230 235	
TAG	675

## (2) INFORMATION FOR SEQUENCE ID NO: 3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 228 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GCATGCAGTT GCAAAGCCCA GAAGAAAGAA ATGGACAGCG GAAGAAGTGG TTGTTTTTT	60
TTCCCTTCA TTAATTTCT AGTTTTAGT AATCCAGAAA ATTTGATTTT GTTCTAAAGT	120
TCATTATGCA AAGATGTCAC CAACAGACTT CTGACTGCAT GGTGAACCTT CATATGATAC	180
ATAGGATTAC ACTTGTACCT GTTAAAATA AAAGTTGAC TTGCATAC	228

## (2) INFORMATION FOR SEQUENCE ID NO: 4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1365 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ACACACAGGAG AATGAAAAGA ACCCGGGACT CCCAAAGACG CTAGATGTGT	50
GAAGATCCTG ATCACTCATT GGGTGTCTGA GTTCTGCGAT ATTCACTCCCT	100
CAGCCAATGA GCTTACTGTT CTCGTGGGGG GTTTGTGAGC CTTGGGTAGG	150
AAGTTTTGCA AGTTCCGCCT ACAGCTCTAG CTTGTGAATT TGTAACCCCTTT	200
CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC CCCCCTCCCCA	250
CCTCGTGTG TGCTGAGTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT	300
AGAACTCTTC CGGAGGAAGG AGGGAGGACC CCCCCCCTTT GCTCTCCAG	350
CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCAAGG AAGTAAGCCG	400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCCTGCTGGT	450
ACCCCTTGTG CC	462
ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA	504
GGT GGT GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG	546
TAC TCC CTG GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC	588
TTC GCT GTT GTC ACA ACA AGT TTT CTG GCG CTC CAG CAT TTC	630
ATA GAC GCC CTT TAT GAG GAG CAG TAT GAA AGG GAT GTG GCC	672
TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC TCT GTC GAT GAG	714
GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC GAC GAC	756
GAG GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT	798
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA	840
GAT GAG GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA	882
GCT GAG GAA ATG GGT GCT GGC GCT AAC TGT GCC TGT GTT CCT	924
GGC CAT CAT TTA AGG AAG AAT GAA GTG AAG TGT AGG ATG ATT	966
TAT TTC TTC CAC GAC CCT AAT TTC CTG GTG TCT ATA CCA GTG	1008
AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT GAA AAT GCT GAT	1050
GAA GAG CTT GCA ATG GAA GAG GAA GAA GAA GAG GAG GAG	1092
GAG GAG GAA GAG GAA ATG GGA AAC CCG GAT GGC TTC TCA CCT	1134
TAG	1137
GCATGCAGTT GCAAAGCCCA GAAGAAAGAA ATGGACAGCG GAAGAAGTGG	1187
TTGTTTTTTT TTCCCTTCA TTAATTTCT AGTTTTAGT AATCCAGAAA	1237
ATTTGATTTT GTTCTAAAGT TCATTATGCA AAGATGTCAC CAACAGACTT	1287
CTGACTGCAT GGTGAACCTT CATATGATAC ATAGGATTAC ACTTGTACCT	1337
GTAAAATA AAAGTTGAC TTGCATAC	1365

## (2) INFORMATION FOR SEQUENCE ID NO: 5:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4698 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: genomic DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ACACACAGGAG	AATGAAAAAGA	ACCCGGGACT	CCCAAAGACG	CTAGATGTGT	50
GAAGATCCTG	ATCACTCATT	GGGTGTCTGA	GTTCTGCGAT	ATTCATCCCT	100
CAGCCAAATGA	GCTTACTGTT	CTCGTGGGGG	GTTTGTGAGC	CTTGGGTAGG	150
AAGTTTTGCA	AGTTCCGCCT	ACAGCTCTAG	CTTGTGAATT	TGTACCCCTT	200
CACGTAAAAAA	AGTAGTCCAG	AGTTTACTAC	ACCCTCCCTC	CCCCCTCCCA	250
CCTCGTGCTG	TGCTGAGTTT	AGAAGTCTTC	CTTATAGAAG	TCTTCCGTAT	300
AGAACTCTTC	CGGAGGAAGG	AGGGAGGAC	CCCCCCCTTT	GCTCTCCCAAG	350
CATGCATTGT	GTCAACGCCA	TTGCACTGAG	CTGGTCGAAG	AAAGTAAGCCG	400
CTAGCTTGCG	ACTCTACTCT	TATCTTAACT	TAGCTCGGCT	TCCTGCTGGT	450
ACCCTTTGTC	CC				462
ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA					504
GGT GGT GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG					546
TAC TCC CTG GAA GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC					588
TTC GCT GTT GTC ACA ACA AGT TTT CTG GCG CTC CAG ATG TTC					630
ATA GAC GCC CTT TAT GAG GAG CAG TAT GAA AGG GAT GTG GCC					672
TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC TCT GTC GAT GAG					714
GAT GAA GAC GAT GAG GAT GAG GAT GAC TAC TAC GAC GAC					756
GAG GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT GAT					798
GAG GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA					840
GAT GAG GCC GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA					882
GCT GAG GAA ATG GGT GCT GCT AAC TGT GCC T					916
GTGACTAACCGTGGCTTT ACTCTAGATT CAGGTGGGTT GCATTCTTTA					966
CTCTTGCCTCA CATCTGTAGT AAAGACCACA TTTTGGTTGG GGGTCATTGC					1016
TGGAGCCATT CCTGGCTCTC CTGTCCACGC CTATCCCCGC TCCTCCCATC					1066
CCCCCACTCCT TGCTCCGCTC TCTTCCCTT TCCCCACCTTG CCTCTGGAGC					1116
TTCAGTCCAT CCTGCTCTGC TCTCCCTTCCCTT GCTCTCTGAT CCCACCCCTC					1166
TCCCCCTCGG CTCAACTTTT CGTGCCTCTC GCTCTCTGAT CCCACCCCTC					1216
TTCAGGCTTC CCCATTGCT CCTCTCCCGA AACCCCTCCCC TTCTGTTCC					1266
CCTTTTCGCG CCTTTCTTTT CCTGCTCCCC TCCCCCTCCC TATTTACCTT					1316
TCACCAGCTT TGCTCTCCCT GCTCCCTCC CCCTTTTGCA CCTTTCTTT					1366
TCCTGCTCCC CTCCCCCTCC CCTCCCTGTT TACCCCTTCAC CGCTTTTCCT					1416
CTACCTGCTT CCCTCCCCCT TGCTGCTCCC TCCCTATTG CATTTCGGG					1466
TGCTCCTCCC TCCCCCTCCC CCTCCCTCCC TATTTGCATT TTGGGGTGCT					1516
CCTCCCTCCC CCTCCCCCAGG CCTTTTTTTT TTTTTTTTTT TTGTTTTTTT					1566
TTGGTTTTTC GAGACAGGGT TTCTCTTTGT ATCCCTGGCT GTCTGGCAC					1616
TCACTCTGTA GACCAGGCTG GCCTCAAAC					1666
CCTCCCAAAT GCTGGGATTA AAGGCTTGCA CCAGGACTGC CCCAGTGCAG					1716
GCCTTTCTT TTTCTCTCTC CTGGTCTCCC TAATCCCTT TCTGCATGTT					1766
AACTCCCCCTT TTGGCACCTT CCCTTACAG GACCCCTCC CCCTCCCTGT					1816
TTCCCTTCCG GCACCCCTCC TAGCCCTGCT CTGTTCCCTC TCCCTGCTCC					1866
CCTCCCCCTC TTTGCTCGAC TTTTAGCAGC CTTACCTCTC CCTGCTTTCT					1916
GCCCGCTTCC CCTTTTTGTG GCCTTTCCCTC CTGGCTCCC TCCACCTTCC					1966
AGCTCACCTT TTTGTTGTT TGTTGTTGTT GTTGTGTTGGT TTGTTTTTT					2016
TTTTTCTGTT GCACCCCTGTT TTCCAAGATC CCCCTCCCCC TCCGGCTTCC					2066
CCTCTGTGTT CCTTTCTGT TCCCTCCCCC TCGCTGGCTC CCCCTCCCTT					2116
TCTGCCTTTC CTGCCCCCTG TCCCTCTCT GCTAACCTTT TAATGCCTTT					2166
CTTTTCTAGA CTCCCCCCTC CAGGCTTGCT GTTGTCTCT GTGCACTTTT					2216
CCTGACCCCTG CTCCCCCTCC CCTCCCCAGCT CCCCTCTT TTCCCACCTC					2266
CCTTTCTCCA GCCTGTCACC CCTCCTTCTC TCCTCTCTGT TTCTCCCACT					2316
TCCTGCTTCC TTTACCCCTT CCCTCTCCCT ACTCTCCCTCC CTGCTGCTG					2366
GACTTCCCTC CCAGCCGCCCG AGTTCCCTGC AGTCCCTGGAG TCTTTCCCTGC					2416
CTCTCTGTC ATCACCTTCC CCTAGTTCA CTTCCCTTTC ACTCTCCCT					2466
ATGTGTCTCT CTTCTATCT ATCCCTTCTT TTCTGTCCCC TCTCTCTGT					2516
CCATCACCTC TCTCTCCCT TCCCTTTCTT CTCTCTTCCA TTTTCTTCCA					2566
CCTGCTTCTT TACCCCTGCCT CTCCCATTGC CCTCTTACCT TTATGCCCAT					2616
TCCATGTCCC CTCTCAATTG CCTGTCCCAT TGTGCTCCCT CACATCTTCC					2666
ATTTCCCTCT TTCTCCCTTA GCCTCTTCTT CCTCTCTCT GTATCTCCC					2716

TTCCCTTTC	TTCTCCCTCC	TCCTTCCCC	TTCCCTATG	CCCTCTACTC	2766
TACTTGATCT	TCTCTCCTCT	CCACATACCC	TTTTCCCTT	CCACCCCTGCC	2816
CTTTGTCCCC	AGACCCCTACA	GTATCCTGTG	CACAGGAAGT	GGGAGGTGCC	2866
ATCAACAACA	AGGAGGCAAG	AAACAGAGCA	AAATCCAAA	ATCAGCAGGA	2916
AAGGCTGGAT	AAAATAAGG	CCAGGTTCTG	AGGACAGCTG	GAATCTAGCC	2966
AAGTGGCTCC	TATAACCCTA	AGTACCAAGG	GAGAAAGTGA	TGGTGAAGTT	3016
CTTGATCCTT	GCTGCTTCTT	TTACATATGT	TGGCACATCT	TTCTCAAATG	3066
CAGGCCATGC	TCCATGCTT	GGCCTGCTC	AGCGTGGTTA	AGTAATGGGA	3116
GAATCTGAAA	ACTAGGGGCC	AGTGGTTTGT	TTTGGGGACA	AATTAGCACG	3166
TAGTGATATT	TCCCCCTAAA	AATTATAACA	AACAGATTCA	TGATTTGAGA	3216
TCCTTCTACA	GGTGAGAAGT	GGAAAAATTG	TCACTATGAA	TTCTTTTTA	3266
GGCTAAAGAT	ACTTGAACC	ATAGAAGCGT	TGTTAAAATA	CTGCTTTCTT	3316
TTGCTAAAT	ATTCTTCTC	ACATATTCA	ATTCTCCAG		3355
GT GTT	CCT	GGC CAT	CAT TTA	AGG AAG	3396
AGG ATG	ATT	TAT	TTC	TC CAC	3438
ATA CCA	GTG	AAC	CCT	AAG GAA	3480
AAT GCT	GAT	GAA	GAG	GTT GCA	3522
GAG GAG	GAG	GAG	GAG	ATG GAA	3564
TTC TCA	CCT	TAG		AAC CCG GAT	3576
GCATGCAGGT	ACTGGCTTCA	CTAACCAACC	ATTCTAACCA	TATGCCTGTA	3626
GCTAAGAGCA	TCTTTTAAA	AAATATTATT	GGTAAACTAA	ACAATTGTTA	3676
TCTTTTACA	TTAATAAGTA	TTAAATTAAAT	CCAGTATACA	TTTTAAAGAA	3726
CCCTAAGTTA	AACACAAAGTC	AATGATGTCT	AGATGCCCTGT	TCTTTAGATT	3776
GTAGTGAGAC	TACTTACTAC	AGATGAGAAG	TTGTTAGACT	CGGGAGTAGA	3826
GACCAGTAA	AGATCATGCA	GTGAAATGTG	GCCATGGAAA	TCGCATATTG	3876
TTCTTATAGT	ACCTTTGAGA	CAGCTGATAA	CAGCTGACAA	AAATAAGTGT	3926
TTCAAGAAAG	ATCACACGCC	ATGGTTACA	TGCAAATTAT	TATTTGTCG	3976
TTCTGATT	TTTCATTTCT	AGACCTGTGG	TTTTAAAGAG	ATGAAAATCT	4026
CTTAAAATT	CCTTCATCTT	TAATTTTCT	TAACTTAGT	TTTTTCACT	4076
TAGAATTCAA	TTCAAATTCT	TAATTCAATC	TTAATTTTTA	GATTTCCTAA	4126
AATGTTTTT	AAAAAAATG	CAAATCTCAT	TTTTAAAGAGA	TGAAAGCAGA	4176
GTAACTGGGG	GGCTTAGGGG	ATCTGTAGGG	TTGCGGTATA	GCAATAGGGA	4226
GTTCTGGCT	CTGAGAAGCA	GTCAGAGAGA	ATGGAAAAC	AGGCCCTTGC	4276
CAGTAGGTTA	GTGAGGTTGA	TATGATCAGA	TTATGGACAC	TCTCCAAATC	4326
ATAAAATACTC	TAACAGCTAA	GGATCTCTGA	GGGAAACACA	ACAGGGAAAT	4376
ATTTTAGTT	CTCCTTGAGA	AAACATGACA	AGACATAAAA	TTGGCAAGAA	4426
AGTCAGGAGT	GTATTCTAAT	AACTGTTGCT	TATCTCTTAT	TTTCTTCTAC	4476
AGTTGCAAG	CCCAGAAGAA	AGAAATGGAC	AGCGGAAGAA	GTGGTTGTTT	4526
TTTTTCCCC	TTCATTAATT	TTCTAGTTT	TAGTAATCCA	AAAAATTGTA	4576
TTTTGTTCTA	AGITCATTA	TGCAAAGATG	TCACCAACAG	ACTTCTGACT	4626
GCATGGTGA	CTTTCATATG	ATACATAGGA	TTACACTTGT	ACCTGTTAAA	4676
AATAAAAGTT	TGACTTGCAT	AC			4698

## (2) INFORMATION FOR SEQUENCE ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Leu Pro Tyr Leu Gly Trp Leu Val Phe

5

## (2) INFORMATION FOR SEQUENCE ID NO: 7:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2418 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GGATCCAGGC	CCTGCCAGGA	AAAATATAAG	GGCCCTGCGT	GAGAACAGAG	50
GGGGTCATCC	ACTGCATGAG	AGTGGGGATG	TCACAGAGTC	CAGCCCACCC	100

TCCTGGTAGC	ACTGAGAAGC	CAGG	CTGTG	CTTGCAGTCT	CACCCCTGAG	150
GGCCCGTGA	TTCCCTCTCC	TGGAGCTCCA	CGAACCCAGGC	AGTGAGGCCT	200	
TGGTCTGAGA	CAGTATCCTC	AGGTACAGA	CGAGAGGATG	CACAGGGTGT	250	
GCCAGCAGTG	AATGTTGCC	CTGAATGCAC	ACCAAGGGCC	CCACCTGCCA	300	
CAGGACACAT	AGGACTCCAC	AGAGTCTGGC	CTCACCTCCC	TACTGTCAGT	350	
CCTGTAGAAT	CGACCTCTGC	TGGCCGGCTG	TACCCCTGAGT	ACCCCTCTCAC	400	
TTCCCTCCCTC	AGGTTTCAG	GGGACAGGCC	AACCCAGAGG	ACAGGATTCC	450	
CTGGAGGGCA	CAGAGGAGCA	CCAAGGAGA	GATCTGTAAG	TAGGCCTTTG	500	
TTAGAGTCTC	CAAGGTTCA	TTCTCAGCTG	AGGCCTCTCA	CACACTCCT	550	
CTCTCCCCAG	GCCTGTGGGT	CTTCATTGCC	CAGCTCCTGC	CCACACTCCT	600	
GCCTGCTGCC	CTGACCGAGAG	TCATCATGTC	TCTTGAGCAG	AGGAGTCTGC	650	
ACTGCAAGCC	TGAGGAAGCC	CTTGAGGCC	AAACAGAGG	CCTGGGCCTG	700	
GTGTGTGTGC	AGGCTGCCAC	CTCCTCCCTC	TCTCCCTCTGG	TCCTGGGCAC	750	
CCTGGAGGAG	GTGCCCACTG	CTGGGTCAAC	AGATCCTCCC	CAGAGTCCCTC	800	
AGGGAGCCTC	CGCCCTTCCC	ACTACCATCA	ACTTCACTCG	ACAGAGGCAA	850	
CCCAGTGGAG	GTTCCACAG	CCGTGAAGAG	GAGGGGCCAA	GCACCTCTTG	900	
TATCCTGGAG	TCCTGTTCC	GAGCAGTA	CACTAAGAAG	GTGGCTGATT	950	
TGGTTGGTT	TCTGCTCCCT	AAATATCGAG	CCAGGGAGCC	AGTCACAAAG	1000	
GCAGAAATGC	TGGAGAGTGT	CATCAAAAT	TACAAGCACT	GTTCCTCTGA	1050	
GATCTTCGGC	AAAGCCTCTG	AGTCCTGCA	GCTGGTCTTT	GGCATTGACG	1100	
TGAAGGAAGC	AGACCCCCACC	GGCCACTCCT	ATGTCCTTGT	CACCTGCCTA	1150	
GGTCTCTCCT	ATGATGGCCT	GCTGGGTGAT	AATCAGATCA	TGCCCAAGAC	1200	
AGGCTTCCTG	ATAATTGTCC	TGGTCATGAT	TGCAATGGAG	GGCGGCCATG	1250	
CTCCGTAGGA	GGAAATCTGG	GAGGAGCTGA	GTGTGATGGA	GGTGTATGAT	1300	
GGGAGGGAGC	ACAGTGCCTA	TGGGGAGCCC	AGGAAGCTGC	TCACCCAAAGA	1350	
TTTGGTGCAG	GAAAAGTACC	TGGAGTACGG	CAGGTGCCGG	ACAGTGATCC	1400	
CGCACGCTAT	GAGTCTCTGT	GGGGTCCAAG	GGCCCTCGCT	GAAACCAGCT	1450	
ATGTGAAAGT	CCTTGAGTAT	GTGATCAAGG	TCAGTGCAAG	AGTTCGCTTT	1500	
TTCTTCCCAT	CCCTGCGTGA	AGCAGCTTTG	AGAGAGGAGG	AAGAGGGAGT	1550	
CTGAGCATGA	GTTGCAGCCA	AGGCCAGTGG	GAGGGGGACT	GGGCCAGTGC	1600	
ACCTTCCAGG	GCCGCGTCCA	GCAGCTCCC	CTGCTCTCGTG	TGACATGAGG	1650	
CCCATTCTTC	ACTCTGAAGA	GAGCGCTCAG	TGTTCTCAGT	AGTAGGTTTC	1700	
TGTTCTATTG	GGTACTTGG	AGATTTATG	TTGTTCTCTT	TTGGAATTGTT	1750	
TCAAATGTTT	TTTTTAAGG	GATGGTTGAA	TGAACCTTCAG	CATCCAAGTT	1800	
TATGAATGAC	AGCAGTCACA	CAGTTCTGTG	TATATAGTTT	AAGGGTAAGA	1850	
GTCTTGTT	TTATTTCAGAT	TGGGAAATCC	ATTCTATTTT	GTGAATTGGG	1900	
ATAATAACAG	CAGTGGAAATA	AGTACTTGA	AATGTGAAAAA	ATGAGCAGTA	1950	
AAATAGATGA	GATAAAGAAC	TAAGAAATT	AAGAGATAGT	CAATTCTTGC	2000	
CTTATACCTC	AGTCTATTCT	GTAAAATT	TAAGATATA	TGCATACCTG	2050	
GATTTCCCTG	GCTTCTTTGA	GAATGTAAGA	GAATTTAAAT	CTGAATAAAG	2100	
AATTCTTCT	GTCCTACTGGC	TCTTTCTTC	TCCATGCACT	GAGCATCTGC	2150	
TTTTTGGAAAG	GCCCTGGGTT	AGTAGTGGAG	ATGCTAAGGT	AAGCCAGACT	2200	
CATACCCACC	CATAAGGGTCG	TAGAGTCTAG	GAGCTGCAGT	CACGTAATCG	2250	
AGGTGGCAAG	ATGTCCTCTA	AAGATGTAGG	GAAAAGTGCAG	AGAGGGGTGA	2300	
GGGTGTGGGG	CTCCGGGTGA	GAGTGGTGA	GTGTCATGC	CCTGAGCTGG	2350	
GGCATTGGG	GCTTTGGGAA	ACTGCAGTTC	CTTCTGGGGG	AGCTGATTGT	2400	
AATGATCTTG	GGTGGATCC				2418	

## (2) INFORMATION FOR SEQUENCE ID NO: 8:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5724 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-1 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CCCGGGGCAC	CACTGGCATC	CCTCCCCCTA	CCACCCCAA	TCCCTCCCTT	50
TACGCCACCC	ATCCAAACAT	CTTCACGCTC	ACCCCCAGCC	CAAGCCAGGC	100
AGAATCCGGT	TCCACCCCTG	CTCTCAACCC	AGGGAAAGCCC	AGGTGCCAG	150
ATGTGACGCC	ACTGACTTGA	GCATTAGTGG	TTAGAGAGAA	GCGAGGTTTT	200
CGGTCTGAGG	GGCGGCTTGA	GATCGGTGGA	GGGAAGCGGG	CCCAGCTCTG	250

TAAGGGAGGCA	AGGTGACATG	CTGAGGGAGG	ACTGAGGACC	CACTTACCCC	300
AGATAGAGCA	CCCCAAATAA	TCCCTTCATG	CCAGTCTGG	ACCATCTGGT	350
GGTGGACTTC	TCAGGCTGGG	CCACCCCCAG	CCCCCTTGC	GCTTAAACCA	400
CTGGGGACTC	GAAGTCAGAG	CTCCGTGTGA	TCAGGGAAGG	GCTGCTTAGG	450
AGAGGGCAGC	GTCCAGGCTC	TGCCAGACAT	CATGCTCAGG	ATTCTCAAGG	500
AGGGCTGAGG	GTCCCCTAAGA	CCCCACTCCC	GTGACCAAC	CCCCACTCCA	550
ATGCTCACTC	CCGTGACCCA	ACCCCCCTCT	CATTGTCATT	CCAACCCCCA	600
CCCCACATCC	CCCACCCCCAT	CCCTCAACCC	TGATGCCAT	CCGCCAGCC	650
ATTCCACCTC	CACCCCCACC	CCACCCCCCA	CGCCCACTCC	CACCCCCCAC	700
CAGGCAGGAT	CCGGTCCCCG	CCAGGAAACA	TCCGGGTGCC	CGGATGTGAC	750
GCCACTGACT	TGCGCATTGT	GGGGCAGAGA	GAAGCGAGGT	TTCCATTCTG	800
AGGGACGGCG	TAGAGTCGG	CCGAAGGAAC	CTGACCCAGG	CTCTGTGAGG	850
AGGCAAGGTG	AGAGGCTGAG	GGAGGACTGA	GGACCCGCC	ACTCCAAATA	900
GAGAGCCCCA	AATATTCCAG	CCCCGCCCTT	GCTGCCAGCC	CTGGCCCACC	950
CGCGGGAAAGA	CGTCTCAGCC	TGGGCTGCC	CCAGACCCCT	GCTCCAAAG	1000
CCTTGAGAGA	CACCAAGGTT	TTCTCCCCAA	GCTCTGAAT	CAGAGGTTGC	1050
TGTGACCCAGG	CCAGGACTGG	TTAGGAGAGG	GCAGGGCACA	GGCTCTGCCA	1100
GGCATCAAGA	TCAGCACCCA	AGAGGGAGGG	CTGTGGGCC	CCAAGACTGC	1150
ACTCCAATCC	CCACTCCAC	CCCATTGCGA	TTCCCATTCC	CCACCCAACC	1200
CCCATCTCCT	CAGCTACACC	TCCACCCCCA	TCCCTACTCC	TACTCCGTCA	1250
CCTGACCACC	ACCCCTCAGC	CCCAGCACCA	GCCCCAACCC	TTCTGCCACC	1300
TCACCCCTCAC	TGCCCCAAC	CCCACCCCTCA	TCTCTCTCAT	GTGCCCAACT	1350
CCCATCGCCT	CCCCCATTCT	GGCAGAATCC	GGTTTGCCCC	TCCTCTCAAC	1400
CCAGGGAAAGC	CCTGGTAGGC	CCGATGTGAA	ACACTGACT	TGAACCTCAC	1450
AGATCTGAGA	GAAGCCAGGT	TCATTTAATG	GTTCTGAGGG	GGGGCTTGAG	1500
ATCCACTGAG	GGGAGTGGTT	TTAGGCTCTG	TGAGGAGGCA	AGGTGAGATG	1550
CTGAGGGAGG	ACTGAGGAGG	CACACACCCC	AGGTAGATGG	CCCCAAATG	1600
ATCCAGTACC	ACCCCTGCTG	CCAGCCCTGG	ACCACCCGGC	CAGGACAGAT	1650
GTCTCAGCTG	GACCACCCCC	CGTCCCGTCC	CACTGCCACT	TAACCCACAG	1700
GGCAATCTGT	AGTCATAGCT	TATGTGACCC	GGGCAGGGTT	GGTCAGGAGA	1750
GGCAGGGCCC	AGGCATCAAG	GTCCAGCATT	CGCCCCGGCAT	TAGGGTCAGG	1800
ACCCCTGGAG	GGAACTGAGG	GTTCCTCACC	CACACCTGTC	TCTCATCTC	1850
CACCGCCACC	CCACTCACAT	TCCCCATACCT	ACCCCTTACC	CCCAACCTCA	1900
TCTTGTCAAGA	ATCCCTGCTG	TCAACCCCACG	GAAGCCACGG	GAATGGCGGC	1950
CAGGCACTCG	GATCTGACG	TCCCCATCCA	GGGTCTGATG	GAGGGAAAGGG	2000
GCTTGAACAG	GGCCTCAGGG	GAGCAGAGGG	AGGGCCTTAC	TGCGAGATGA	2050
GGGAGGCCTC	AGAGGACCCA	GCACCCCTAGG	ACACCGCACC	CCTGTCTGAG	2100
ACTGAGGCTG	CCACTCTCTGG	CCTCAAGAAT	CAGAACGATG	GGGACTCAGA	2150
TTGCATGGGG	GTGGGACCCA	GGCCTGCAAG	GCTTACGCGG	AGGAAGAGGA	2200
GGGGAGGACTC	AGGGGACCTT	GGAAATCCAGA	TCAGTGTGGA	CCTCGGCCCT	2250
GAGAGGTCCA	GGGCACGGGT	GCCACATATG	GCCCATATTT	CCTGCATCTT	2300
TGAGGTGACA	GGACAGAGCT	GTGGTCTGAG	AAAGTGGGCC	TCAGGTCAAC	2350
AGAGGGAGGA	GTTCCAGGAT	CCATATGGCC	CAAGATGTGC	CCCCTCATG	2400
AGGACTGGGG	ATATCCCCGG	CTCAGAAAGA	AGGGACTCCA	CACAGTCTGG	2450
CTGTCCTCTT	TTAGTAGCTC	TAGGGGACC	AGATCAGGG	TGGCGGTATG	2500
TTCCATTCTC	ACTTGTAACC	CAGGCAGGAA	GTGGGGGGCC	CCTCAGGGAG	2550
ATGGGGTCTT	GGGGTAAAGG	GGGGATGTCT	ACTCATGTCA	GGGAATTGGG	2600
GGTTGAGGAA	GCACAGGCC	TGGCAGGAAT	AAAGATGAGT	GAGACAGACA	2650
AGGCTATTGG	ATTCACACC	CCAGAACCAA	AGGGCTCAGC	CCTGGACACC	2700
TCACCCAGGA	TGTGGCTTCT	TTTCACTCC	TGTTTCCAGA	TCTGGGGCAG	2750
GTGAGGACTC	CATTCTCAGA	GGGTGACTCA	GGTCAACGTA	GGGACCCCCA	2800
TCTGGTCTAA	AGACAGAGCG	GTCCCAGGAT	CTGCCATGCG	TTCGGGTGAG	2850
GAACATGAGG	GAGGACTGAG	GGTACCCAG	GACCAGAAC	CTGAGGGAGA	2900
CTGCACAGAA	ATCAGCCCTG	CCCCCTGCTG	CACCCACAG	AGCATGGGCT	2950
GGGCCGCTG	CCGAGGTCTC	TCCCTTATCC	TGGGATCATT	GATGTCAGGG	3000
ACGGGGAGGC	CTTGGCTGTA	GAAGGCTGG	CTCAGGTCAG	TAGAGGGAGC	3050
GTCCCCAGGCC	CTGCCAGGAG	TCAAGGTGAG	GACCAAGCGG	GCACCTCACC	3150
CAGGACACAT	TAATTCCAAT	GAATTTGAT	ATCTCTTGCT	GCCCCCTCCCC	3200
AAGGACCTAG	GCACGTGTGG	CCAGATGTT	GTCCCCCTCC	GTCTTCCAT	3250
TCCTTATCAT	GGATGTGAAC	TCTTGATTG	GATTTCAG	ACCAGCAAA	3300
GGGCAGGATC	CAGGCCCTGC	CAGGAAAAT	ATAAGGGCCC	TGCGTGAGAA	3350
CAGAGGGGGT	CATCCACTGC	ATGAGAGTGG	GGATGTCACA	GAGTCCAGCC	3400
CACCCCTCTG	GTAGCACTGA	GAAGCCAGGG	CTGTGCTTGC	GGTCTGCACC	3450
CTGAGGGCCC	GTGGATTCTC	CTTCCCTGGAG	CTCCAGGAAC	CAGGCAGTGA	3500
GGCCTTGGTC	TGAGACAGTA	TCCTCAGGTC	ACAGAGCAGA	GGATGCACAG	3550
GGTGTGCCAG	CAGTGAATGT	TTGCCCTGAA	TGCACACCAA	GGGCCCCACC	3600

TGCCACAGGA	CACATAGGAC	TCCACAGAGT	CTGGCCTCAC	CTCCCTACTG	3650
TCAGTCCTGT	AGAATCGACC	TCTGCTGGCC	GGCTGTACCC	TGAGTACCCCT	3700
CTCACTTCCT	CCTTCAGGTT	TTCAGGGGAC	AGGCCAACCC	AGAGGACAGG	3750
ATTCCTGGA	GGCCACAGAG	GAGCACCAAG	GAGAAGATCT	GTAAGTAGGC	3800
CTTTGTTAGA	GTCTCCAAGG	TTCAGTTCTC	AGCTGAGGCC	TCTCACACAC	3850
TCCCTCTCTC	CCCAGGCCCTG	TGGGTCTTC	TTGCCAGCT	CCTGCCACACA	3900
CTCCGTGCTG	CTGCCCTGAC	GAGAGTCATC			3930
ATG TCT CTT	GAG CAG AGG	AGT CTG CAC	TGC AAG CCT	GAG GAA	3972
GCC CTT GAG	GCC CAA CAA	GAG GCC CTG	GTC TGT GTG	TGT GTG	4014
CAG GCT GCC	ACC TCC TCC	TCC TCT CCT	CTG GTC CTG	GTC ACC	4056
CTG GAG GAG	GTG CCC ACT	GCT GGG TCA	ACA GAT CCT	CCC CAG	4098
AGT CCT CAG	GGA GCC TCC	GCC TTT CCC	ACT ACC ATC	AAC TTC	4140
ACT CGA CAG	AGG CAA CCC	AGT GAG GGT	TCC AGC AGC	CGT GAA	4182
GAG GAG GGG	CCA AGC ACC	TCT TGT ATC	CTG GAG TCC	TTG TTC	4224
CGA GCA GTA	ATC ACT AAG	AAG GTG GCT	GAT TTG GTT	GGT TTT	4266
CTG CTC CTC	AAA TAT CGA	GCC AGG GAG	CCA GTC ACA	AAG GCA	4308
GAA ATG CTG	GAG AGT GTC	ATC AAA AAT	TAC AAG AAC	CAC TGT	4350
CCT GAG ATC	TTC GGC AAA	GCC TCT GAG	TCC TTG CAG	CTG GTC	4392
TTT GGC ATT	GAC GTG AAG	GAA GCA GAC	CCC ACC GGC	CAC TCC	4434
TAT GTC CTT	GTC ACC TGC	CTA GGT CTC	TCC TAT GAT	GGC CTG	4476
CTG GGT GAT	AAT CAG ATC	ATG CCC AAG	ACA GGC TTC	CTG ATA	4518
ATT GTC CTG	GTC ATG ATT	GCA ATG GAG	GGC GGC CAT	GCT CCT	4560
GAG GAG GAA	ATC TGG GAG	GAG CTG AGT	GTG ATG GAG	GTG TAT	4602
GAT GGG AGG	GAG CAC AGT	GCC TAT GGG GAG	CCC AGG AAG	CTG CTG	4644
CTC ACC CAA GAT	TTG GTG CAG	GAA AAG TAC	CTG GAG TAC	GGC GGC	4686
AGG TGC CGG	ACA GTG ATC	CCG CAC GCT	ATG AGT TCC	TGT GGG	4728
GTC CAA GGG	CCC TCG CTG	AAA CCA GCT	ATG TGA		4761
AAGTCCTTGA	GTATGTGATC	AAGGTCAGTG	CAAGAGTTC		4800
GCTTTTTCTT	CCCATCCCTG	CGTGAAGCAG	CTTGAGAGA	GGAGGAAGAG	4850
GGAGTCTGAG	CATGAGTTGC	AGCCAAGGCC	AGTGGGAGGG	GGACTGGGCC	4900
AGTGCACCTT	CCAGGGCCGC	GTCCAGCAGC	TTCCCTGCC	TCGTGTGACA	4950
TGAGGCCCAT	TCTTCACTCT	GAAGAGAGCG	GTCAGTGTTC	TCAGTAGTAG	5000
GTTCCTGTT	TATTGGGTGA	CTTGGAGATT	TATCTTGTT	CTCTTTGGA	5050
ATTGTTCAAA	TGTTTTTTT	TAAGGGATGG	TTGAATGAAC	TTCAGCATCC	5100
AAGTTTATGA	ATGACAGCAG	TCACACAGTT	CTGTGTATAT	AGTTTAAGGG	5150
TAAGAGTCTT	GTGTTTTATT	CAGATTGGGA	AATCCATTCT	ATTTGTGAA	5200
TTGGGATAAT	AACAGCAGTG	GAATAAGTAC	TTAGAAATGT	AAAAAATGAG	5250
CAGTAAATA	GATGAGATAA	AGAACTAAAG	AAATTAAGAG	ATAGTCATT	5300
CTTGCCTTAT	ACCTCAGTCT	ATTCTGTAAA	ATTTTAAAG	ATATATGCAT	5350
ACCTGGATT	CTTGGCTTC	TTTGAGAAT	TAAGAGAAAT	TAATCTGAA	5400
TAAAGAATTC	TTCCTGTTCA	CTGGCTCTTT	TCTTCTCCAT	GCACGTGAGCA	5450
TCTGCTTTT	GGAAAGCCCT	GGGTTAGTAG	TGGAGATGCT	AAGGTAAGCC	5500
AGACTCATAC	CCACCCATAG	GGTCGTAGAG	TCTAGGAGCT	GCAGTCACGT	5550
AATCGAGGTG	GCAAGATGTC	CTCTAAACAT	GTAGGGAAAA	GTGAGAGAGG	5600
GGTGAGGGTG	TGGGGCTCCG	GGTGAGAGTG	GTGGAGTGTC	AATGCCCTGA	5650
GCTGGGGCAT	TTTGGGCTTT	GGGAAACTGC	AGTTCCCTCT	GGGGGAGCTG	5700
ATTGTAATGA	TCTTGGGTGG	ATCC			5724

## (2) INFORMATION FOR SEQUENCE ID NO: 9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4157 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: genomic DNA

## (ix) FEATURE:

- (A) NAME/KEY: MAGE-2 gene

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CCCCATCCAGA	TCCCCATCCG	GGCAGAAATCC	GGTTCCACCC	TTGCCGTGAA	50
CCCAGGGAAAG	TCACGGGCCC	GGATGTGACG	CCACTGACTT	GCACATTGGA	100
GGTCAGAGGA	CAGCGAGATT	CTCGCCCTGA	GCAACGGCCT	GACGTCGGCG	150
GAGGGAAAGCA	GGCGCAGGCT	CCGTGAGGAG	GCAAGGTAAG	ACGCGCAGGG	200
AGGACTGAGG	CGGGCCTCAC	CCCAGACAGA	GGGCCCCCAA	TAAATCCAGC	250

GCTGCCTCTG	CTGCCGGGCC	TGGACCACCC	TGCAGGGAA	GACTTCTCAG	300
GCTCAGTCGC	CACCACTCA	CCCCGCCAAC	CCCCGGCGCT	TTAACCGCAG	350
GGAACCTCTGG	CGTAAGAGCT	TTGTGTGACC	AGGGCAGGGC	TGTTAGAAG	400
TGCTCAGGGC	CCAGACTCAG	CCAGGAATCA	AGGTCAAGGAC	CCCAAGAGGG	450
GACTGAGGGC	AACCCACCCC	CTACCCCTCAC	TACCAATCCC	ATCCCCCAAC	500
ACCAACCCCCA	CCCCCATCCC	TCAAACACCA	ACCCACCCCC	CAAACCCCCAT	550
TCCCCTCTCC	TCCCCCACCA	CCATCCTGGC	AGAATCCGGC	TTTGGCCCCCTG	600
CAATCAACCC	ACGGAAGCTC	GGGGAATGGC	GGCCAAAGCAC	GCGGATCCTG	650
ACGTTCACAT	GTACGGCTAA	GGGAGGGAAAG	GGGTTGGGTC	TCGTGAGTAT	700
GGCCTTTGGG	ATGCAGAGGA	ACGGGCCAGG	CCTCCTGGAA	GACAGTGGAG	750
TCCTTAGGGG	ACCCAGCATG	CCAGGACAGG	GGGCCCCACTG	TACCCCTGTC	800
TCAAACGTAG	CCACCTTTTC	ATTCAAGCCGA	GGGAATCCTA	GGGATGCAGA	850
CCCACCTTCAG	GGGGTTGGGG	CCCAGCCTGC	GAGGAGTCAA	GGGGAGGAAG	900
AAGAGGGAGG	ACTGAGGGGA	CCTTGGAGTC	CAGATCACTG	GCAACCTTGG	950
GCTGGGGGAT	CCTGGGCACA	GTGGCCGAAT	GTGCCCCGTG	CTCATTGCAC	1000
CTTCAGGGT	ACAGAGAGT	GAGGGCTGTG	GTCTGAGGGC	TGGGACTTCA	1050
GTCAGCAGA	GGGAGGAATC	CCAGGATCTG	CCGGACCCCA	GGTGTGCCCC	1100
CTTCATGAGG	ACTCCCCATA	CCCCCGGCC	AGAAAGAAGG	GATGCCACAG	1150
AGTCTGGAAAG	AAATTGTT	TTAGCTCTGG	GGGAACCTGA	TCAGGGATGG	1200
CCCTAAGTGA	CAATCTCATT	TGTACCACAG	GCAGGAGGTT	GGGAAACCCCT	1250
CAGGGAGATA	AGGTGTTGGT	GTAAAGAGGA	GCTGTCTGCT	CATTTCAAGGG	1300
GGTTCCCCCT	TGAGAAAGGG	CAGTCCCTG	CAGGAGTAAA	GATGAGTAAC	1350
CCACAGGGAGG	CCATCATAAAC	GTTCACCCCTA	GAACCAAAAGG	GGTCAGCCCT	1400
GGACAAACGCA	CCTGGGGTAA	CAGGATGTGG	CCCCCTCTCA	CTTGTCTTTC	1450
CAGATCTCAG	GGAGTTGATG	ACCTTGTGTT	CAGAAGGTGA	CTCAGTCAAC	1500
ACAGGGCCCCC	CTCTGGTCGA	CACATGCAGT	GGTTCTAGGA	TCTGCCAACG	1550
ATCCAGGTGG	AGAGCCTGAG	GTAGGATTGA	GGGTACCCCT	GGGCCAGAAT	1600
GCAGCAAGGG	GGCCCCATAG	AAATCTGCC	TGCCCCCTGCG	GTTACTTCAG	1650
AGACCCCTGGG	CAGGGCTGTC	ACCTGAAGTC	CCTCCATTAT	CTGGGATCTT	1700
TGATGTCAGG	GAAGGGGGAGG	CCTTGGTCTG	AAGGGGCTGG	AGTCAGGTCA	1750
GTAGAGGGAGG	GGTCTCAGGG	CTGCCAGGA	GGGACCTGTA	GGACCAAGCG	1800
GACTCGTCAC	CCAGGACACCC	TGGACTCCAA	TGAATTGAC	ATCTCTCGTT	1850
GTCTCTCCG	GAGGACCTGG	TCACGTATGG	CCAGATGTGG	GTCCCCCTCTA	1900
TCTCCTCTG	TACCATATCA	GGGATGTGAG	TTCTTGACAT	GAGAGATTCT	1950
CAAGCCAGCA	AAAGGGTGGG	ATTAGGCCCT	ACAAGGAGAA	AGGTGAGGGC	2000
CCTGAGTGGAG	CACAGAGGGG	ACCCCTCCAC	CAAGTAGAGT	GGGGACCTCA	2050
CGGAGCTCTGG	CCAACCTTC	TGAGACTCTT	GGGAATCCGT	GGCTGTGCTT	2100
GCAGTCTGCA	CACTGAAGGC	CCGTGCATTTC	CTCTCCCAAGG	AATCAGGAGC	2150
TCCAGGAACCC	AGGCAGTGGAG	GCCCTTGGTCT	GAGTCAGTGC	CTCAGGTAC	2200
AGAGCAGAGG	GGACGCAGAC	AGTGCCAAC	CTGAAGGTTT	GCCTGGAATG	2250
CACACCAAGG	CCCCCACCCG	CCCAGAACAA	ATGGGACTCC	AGAGGGCCTG	2300
GCCTCACCCCT	CCCTATTCTC	AGTCCTGAG	CCTGAGCATG	TGCTGGCCGG	2350
CTGTACCCCTG	AGGTGCCCTC	CCACTTCCCTC	CTTCAGGTTTC	TGAGGGGGAC	2400
AGGCTGACAA	GTAGGACCCG	ACGCACTGGA	GGAGCATTGA	AGGAGAAAGAT	2450
CTGTAAAGTAA	GCCTTGTCA	GAGCCTCCAA	GGTTGAGCTTC	AGTTCTCACC	2500
TAAGGGCTCA	CACACCCCTC	TTCTCTCCCC	AGGCCTGTGG	GTCTTCATTG	2550
CCCAGCTCCT	CCCCGCACTC	CTGCCCTGCTG	CCCTGACCG	AGTCATC	2597
ATG CCT CTT GAG CAG AGG ACT CAG CAC TGC AAG CCT GAA GAA					2639
GGC CTT GAG GCC CGA GGA GAG GCC CTG GGC CTG GTG GGT GCG					2681
CAG GCT CCT GCT ACT GAG GAG CAG CAG ACC GCT TCT TCC TCT					2723
TCT ACT CTA GTG GAA GTT ACC CTG GGG GAG GTG CCT GCT GCC					2765
GAC TCA CCG AGT CCT CCC CAC AGT CCT CAG GGA GCC TCC AGC					2807
TTC TCG ACT ACC ATC AAC TAC ACT CTT TGG AGA CAA TCC GAT					2849
GAG GGC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGA ATG TTT					2891
CCC GAC CTG GAG TCC GAG TTC CAA GCA GCA ATC AGT AGG AAG					2933
ATG GTT GAG TTG GTT CAT TTT CTG CTC CTC AAG TAT CGA GCC					2975
AGG GAG CCG GTC ACA AAG GCA GAA ATG CTG GAG AGT GTC CTC					3017
AGA AAT TGC CAG GAC TTC TTT CCC GTG ATC TTC AGC AAA GCC					3059
TCC GAG TAC TTG CAG CTG GTC TTT GGC ATC GAG GTG GTG GAA					3101
GTG GTC CCC ATC AGC CAC TTG TAC ATC CTT GTC ACC TGC CTG					3143
GGC CTC TCC TAC GAT GGC CTG CTG GGC GAC AAT CAG GTC ATG					3185
CCC AAG ACA GGC CTC CTG ATA ATC GTC CTG GCC ATA ATC GCA					3227
ATA GAG GGC GAC TGT GCC CCT GAG GAG AAA ATC TGG GAG GAG					3269
CTG AGT ATG TTG GAG GTG TTT GAG GGG AGG GAG GAC AGT GTC					3311
TTC GCA CAT CCC AGG AAG CTG CTC ATG CAA GAT CTG GTG CAG					3353
GAA AAC TAC CTG GAG TAC CGG CAG GTG CCC GGC AGT GAT CCT					3395

GCA TGC TAC GAG TTC CTG TGG GGT CCA AGG GCC CTC ATT GAA	3437
ACC AGC TAT GTG AAA GTC CTG CAC CAT ACA CTA AAG ATC CGT	3479
GGA GAA CCT CAC ATT TCC TAC CCA CCC CTG CAT GAA CGG GCT	3521
TTG AGA GAG GGA GAA GAG TGA	3542
GTCTCAGCAC ATGTTGCAGC CAGGGCCAGT GGGAGGGGT CTGGGCCAGT	3592
GCACCTTCCA GGGCCCCATC CATTAGCTTC CACTGCCCTCG TGTGATATGA	3642
GGCCCATTCC TGCCTCTTTC AAGAGAGCAG TCAGCATTCT TAGCAGTGAG	3692
TTTCTGTTCT GTTGGATGAC TTGAGATT ATCTTCTTT CCTGTTGGAA	3742
TTGTTCAAAT GTTCCCTTTA ACAAAATGGTT GGATGAACCT CAGCATCCAA	3792
GTTTATGAAT GACAGTAGTC ACACATAGTG CTGTTTATAT AGTTTACGGG	3842
TAAGAGTCCT GTTTTTTATT CAGATTGGGA AATCCATTCC ATTTTGTGAG	3892
TTGTCACATA ATAACAGCAG TCCAATATGT ATTTGCCAT ATTGTGAACG	3942
AATTAGCAGT AAAATACATG ATACAAGGAA CTCAAAAGAT AGTTAATTCT	3992
TGCCTTATAC CTCAGTCTAT TATGTAATAAT TAAAAAATATG TGATGTTTT	4042
TGCTTCTTTG AGAATGCAAAG AGAAATTAAA TCTGAATAAA TTCTTCCTGT	4092
TCACGGGCTC ATTTCTTTAC CATTCACTCA GCATCTGCTC TGTGGAAGGC	4142
CCTGGTAGTA GTGGG	4157

## (2) INFORMATION FOR SEQUENCE ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 662 base pairs
  - (B) TYPE: nucleic acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (ix) FEATURE:
  - (A) NAME/KEY: MAGE-21 gene
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGATCCCCAT GGATCCAGGA AGAATCCAGT TCCACCCCTG CTGTGAACCC	50
AGGGAAAGTCA CGGGGCCGGA TGTGACGCCA CTGACTTGCG CGTTGGAGGT	100
CAGAGAACAG CGAGATTCTC GCCCCTGAGCA ACGGCCCTGAC GTCGGGCGGAG	150
CGAGGCAGGC GCAGGCTCCG TGAGGAGGCA AGGTAAAGATG CCGAGGGAGG	200
ACTGAGGGCG GCCTCACCCCC AGACAGAGGG CCCCCAATAA TCCAGCGCTG	250
CCTCTGCTGC CAGGCCCTGGA CCACCCCTGCA GGGGAAGACT TCTCAGGCTC	300
AGTCGCCACC ACCTCACCCCC GCCACCCCCC GCGCCTTAA CCGCAGGGAA	350
CTCTGGTGTGTA AGAGCTTGT GTGACCAGGG CAGGGCTGGT TAGAAGTGCT	400
CAGGGCCCAAG ACTCAGCCAG GAATCAAGGT CAGGACCCCCA AGAGGGGACT	450
GAGGGTAACC CCCCCCGCACC CCCACCCACCA TTCCCCATCCC CCAACACCAA	500
CCCCACCCCCC ATCCCCAAC ACCAAACCCA CCACCATCGC TCAAACATCA	550
ACGGCACCCCCA CAAACCCCCGA TTCCCCATCCC CACCCATCCT GGCAGAAATCG	600
GAGCTTGCC CCTGCAATCA ACCCACCGAA GCTCCGGAA TGGCGGCCAA	650
GCACCGGGAT CC	662

## (2) INFORMATION FOR SEQUENCE ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1640 base pairs
  - (B) TYPE: nucleic acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA
- (ix) FEATURE:
  - (A) NAME/KEY: cDNA MAGE-3
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GCCCGCAGGG AAGCCGGCCC AGGCTCGGTG AGGAGGCAAG GTTCTGAGGG	50
GACAGGCTGA CCTGGAGGAC CAGAGGCCCG CGGAGGACCA CTGAAGGAGA	100
AGATCTGCCA GTGGGTCTCC ATTGCCAGC TCCTGCCAC ACTCCCGCCT	150
GTTGCCCTGA CCAGAGTCAT C	171
ATG CCT CTT GAG CAG AGG AGT CAG CAC TGC AAG CCT GAA GAA	213
GGC CTT GAG GCC CGA GGA GAG GCC CTG GGC CTG GTG GGT GCG	255
CAG GCT CCT GCT ACT GAG GAG CAG GAG GCT GCC TCC TCC TCT	297
TCT ACT CTA GTT GAA GTC ACC CTG GGG GAG GTG CCT GCT GCC	339

GAG TCA CCA GAT CCT CCC CAG AGT CCT CAG GGA GCC TCC AGC	381
CTC CCC ACT ACC ATG AAC TAC CCT CTC TGG AGC CAA TCC TAT	423
GAG GAC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGC ACC TTC	465
CCT GAC CTG GAG TCC GAG TTC CAA GCA GCA CTC AGT AGG AAG	507
GTG GCC GAG TTG GTT CAT TTT CTG CTC CTC AAG TAT CGA GCC	549
AGG GAG CCG GTC ACA AAG GCA GAA ATG CTG GGG AGT GTC GTC	591
GGA AAT TGG CAG TAT TTC TTT CCT GTG ATC TTC AGC AAA GCT	633
TCC AGT TCC TTG CAG CTG GTC TTT GGC ATC GAG CTG ATG GAA	675
GTG GAC CCC ATC GGC CAC TTG TAC ATC TTT GCC ACC TGC CTG	717
GGC CTC TCC TAC GAT GGC CTG CTG GGT GAC AAT CAG ATC ATG	759
CCC AAG GCA GGC CTC CTG ATA ATC GTC CTG GCC ATA ATC GCA	801
AGA GAG GGC GAC TGT GCC CCT GAG GAG AAA ATC TGG GAG GAG	843
CTG AGT GTG TTA GAG GTG TTT GAG GGG AGG GAA GAC AGT ATG	885
TTG GGG GAT CCC AAG AAG CTG CTC ACC CAA CAT TTC GTG CAG	927
GAA AAC TAC CTG GAG TAC CGG CAG GTC CCC GGC AGT GAT CCT	969
GCA TGT TAT GAA TTC CTG TGG GGT CCA AGG GCC CTC GTT GAA	1011
ACC AGC TAT GTG AAA GTC CTG CAC CAT ATG GTA AAG ATC AGT	1053
GGA GGA CCT CAC ATT TCC TAC CCA CCC CTG CAT GAG TGG GTT	1095
TTG AGA GAG GGG GAA GAG TGA	1116
GTCTGAGCAC GAGTTGCCAGC CAGGGCCAGT GGGAGGGGGT CTGGGCCAGT	1166
GCACCCCTCCG GGGCCGCATC CCTTAGTTTC CACTGCCTCC TGTGACGTGA	1216
GGCCCCATTCT TCACTCTTTG AAGCGAGCAG TCAGCATTCT TAGTAGTGGG	1266
TTTCTGTTCT GTTGGATGAC TTGAGATTA TTCTTTGTT CCTGTTGGAG	1316
TTGTTCAAAT GTTCCCTTTA ACGGATGGTT GAATGAGCCT CAGCATCCAG	1366
GTTTATGAAT GACAGTAGTC ACACATAGTC CTGTTTATAT AGTTTAGGAG	1416
TAAGACTCTT GttTTTACT CAAATTggGA AATCCATTCC ATTGTTGAA	1466
TTGTGACATA ATAATAGCAG TGGTAAAAGT ATTGCTTAA AATTGTTGAGC	1516
GAATTAGCAA TAACATACAT GAGATAACTC AAGAAATCAA AAGATAGTTG	1566
ATTCTTGCTC TGTACCTCAA TCTATTCTGT AAAATTAAAC AAATATGCAA	1616
ACCAGGATTTC CCTTGACTTC TTG	1640

## (2) INFORMATION FOR SEQUENCE ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 943 base pairs
  - (B) TYPE: nucleic acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (ix) FEATURE:
  - (A) NAME/KEY: MAGE-31 gene
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGATCCTCCA CCCCCAGTAGA GTGGGGACCT CACAGAGTCT GCCCAACCCCT	50
CCTGACAGTT CTGGGAATCC GTGGCTGCGT TTGCTGTCTG CACATTGGGG	100
CCCCGTTGGAT TCCTCTCCCA GGAATCAGGA GCTCCAGGAA CAAGGCAGTG	150
AGGACTTGGT CTGAGGCAGT GTCCTCAGGT CACAGAGTAG AGGGGGCTCA	200
GATAGTGCCA ACGGTGAAGG TTGCTCTGG ATTCAAACCA AGGGCCCCCAC	250
CTGCCCCAGA ACACATGGAC TCCAGAGCAG CTGGCCCTCAC CCTCAAACT	300
TTCAGTCTCG CAGCCTCAGC ATGCGCTGGC CGGATGTACC CTGAGGTGCC	350
CTCTCACTTC CTCCCTCAGG TTCTGAGGGG ACAGGCTGAC CTGGAGGACC	400
AGAGGCCCCC GGAGGAGCAC TGAAGGAGAA GATCTGTAAG TAAGCCTTG	450
TTAGAGCCTC CAAGGTTCCA TTCAGTACTC AGCTGAGGTC TCTCACATGC	500
TCCCCTCTCTC CCCAGGCCAG TGGGTCTCCA TTGCCAGCT CCTGCCACAA	550
CTCCCGCCCTG TTGCCCTGAC CAGAGTCATC	580
ATG CCT CTT GAG CAG AGG AGT CAG CAC TGC AAG CCT GAA GAA	622
GTC CTT GAG GCC CGA GGA GAG GCC CTG GGC CTG GTG GGT GCG	664
CAG GCT CCT GCT ACT GAG GAG CAG GAG GGT GCC TCC TCC TCT	706
TCT AGT GTA GTT GAA GTC ACC CTG GGG GAG GTG CCT GCT GCC	748
GAG TCA CCA GAT CCT CCC CAG AGT CCT CAG GGA GCC TCC AGC	790
CTC CCC ACT ACC ATG AAC TAC CCT CTC TGG AGC CAA TCC TAT	832
GAG GAC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGC ACC TTC	874
CCT GAC CTG GAG TCT GAG TTC CAA GCA GCA CTC AGT AGG AAG	916
GTG GCC AAG TTG GTT CAT TTT CTG CTC	943

## (2) INFORMATION FOR SEQUENCE ID NO: 13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2531 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: genomic DNA

## (ix) FEATURE:

- (A) NAME/KEY: MAGE-4 gene

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GGATCCAGGC	CCTGCCTGGA	GAAATGTGAG	GGCCCTGAGT	GAACACAGTG	50
GGGATCATCC	ACTCCATGAG	AGTGGGGAC	TCACAGAGTC	CAGCCTACCC	100
TCTTGATGGC	ACTGAGGGAC	CGGGGCTGTG	CTTACAGTCT	GCACCCCTAAG	150
GGCCCATGGA	TTCCCTCTCT	AGGAGCTCCA	GGAAACAAAGGC	AGTGAGGCCT	200
TGGTCTGAGA	CAGTGTCTC	AGGTTACAGA	GCAGAGGATG	CACAGGCTGT	250
GCCAGCAGTG	AATGTTGCG	CTGAATGCAC	ACCAAGGGCC	CCACCTGCCA	300
CAAGACACAT	AGGACTCCAA	AGAGTCTGGC	CTCACCTCCC	TACCATCAAT	350
CCTGCAGAAT	CGACCTCTGC	TGGCCGGCTA	TACCCCTGAGG	TGCTCTCTCA	400
CTTCCTCCTT	CAGGTTCTGA	GCAGACAGGC	CAACCGGAGA	CAGGATTCCC	450
TGGAGGCCAC	AGAGGAGCAC	CAAGGAGAAAG	ATCTGTAAGT	AAGCCTTTGT	500
TAGAGCCTCT	AAGATTGGT	TCTCAGCTGA	GGTCTCTCAC	ATGCTCCCTC	550
TCTCCGTAGG	CCTGTGGGT	CCCATTTGCC	AGCTTTGCC	TGCACTCTTG	600
CCTGCTGCC	TGACCAGAGT	CATC			624
ATG TCT TCT	GAG CAG AAG	AGT CAG CAC	TGC AAG CCT	GAG GAA	666
GTC GTT GAG	GCC CAA GAA	GAG GCC	CTG GGC	CTG GTG GGT	708
CAG GCT CCT	ACT ACT GAG	GAG CAG GAG	GCT GCT	GTC TCC TCC	750
TCC TCT CCT	CTG GTC CCT	GGC ACC CTG	GAG GAA GTG	CCT GCT	792
GCT GAG TCA	GCA GGT CCT	CCC CAG AGT	CCT CAG GGA	GCA GCC TCT	834
GCC TTA CCC	ACT ACC ATC	AGC TTC ACT	TGC TGG AGG	CAA CCC	876
AAT GAG GGT	TCC AGC AGC	CAA GAA GAG	GAG GGG CCA	AGC ACC	918
TCG CCT GAC	GCA GAG TCC	TTG TTC CGA	GAA GCA CTC	AGT AAC	960
AAG GTG GAT	GAG TTG GCT	CAT TTT CTG	CTC CGC AAG	TAT CGA	1002
GCC AAG GAG	CTG GTC ACA	AAG GCA GAA	ATG CTG GAG	AGA GTC	1044
ATC AAA AAT	TAC AAG CGC	TGC TTT CCT	GTG ATC TTC	GGC AAA	1086
GCC TCC GAG	TCC CTG AAG	ATG ATC TTT	GGC ATT GAC	GTG AAG	1128
GAA GTG GAC	CCC GCC AGC	AAC ACC TAC	ACC CTT GTC	ACC TGC	1170
CTG GGC CTT	TCC TAT GAT	GGC CTG CTG	GGT AAT AAT	CAG ATC	1212
TTT CCC AAG	ACA GGC CTT	CTG ATA ATC	GTC CTG GGC	ACA ATT	1254
GCA ATG GAG	GGC GAC AGC	GCC TCT GAG	GAG GAA ATC	TGG GAG	1296
GAG CTG GGT	GTG ATG GGG	GTG TAT GAT	GGG AGG GAG	CAC ACT	1338
GTC TAT GGG	GAG CCC AGG	AAA CTG CTC	ACC CAA GAT	TGG GTG	1380
CAG GAA AAC	TAC CTG GAG	TAC CGG CAG	GTA CCC GGC	AGT AAT	1422
CCT GCG CGC	TAT GAG TTC	CTG TGG GGT	CCA AGG GCT	CTG GCT	1464
GAA ACC AGC	TAT GTG AAA	GTC CTG GAG	CAT GTG GTC	AGG GTC	1506
AAT GCA AGA	GTT CGC ATT	GCC TAC CCA	TCC CTG CGT	GAA GCA	1548
GCT TTG TTA	GAG GAA GAG	GGG GGA GTC	TGA		1578
GCATGACTTG	CAGCCAGGGC	TGTGGGAAAG	GGGCAGGGCT	GGGCCAGTGC	1628
ATCTAACAGC	CCTGTGCAGC	AGCTTCCCTT	GCCTCGTGA	ACATGAGGCC	1678
CATTCTCAC	TCTGTTGAA	AAAAATAGTC	AGTGTCTTA	GTAGTGGTT	1728
TCTATTTGTT	TGGATGACTT	GGAGATTTAT	CTCTGTTCC	TTTACAAATT	1778
GTTGAAATGT	TCCTTTAAT	GGATGGTTGA	ATTAACCTCA	GCATCCAAGT	1828
TTATGAATCG	TAGTTAACGT	ATATTGCTGT	TAATATAGTT	TAGGAGTAAG	1878
AGTCTTGT	TTTATTTCAGA	TTGGGAAATC	CGTTCTATT	TGTGAATTG	1928
GGACATAATA	ACAGCAGTGG	AGTAAGTATT	TAGAAGTGTG	AATTCCACCGT	1978
GAAATAGGTG	AGATAAAATT	AAAGATACTT	AATTCCCGCC	TTATGCCCTCA	2028
GTCTATTCTG	AAAAATTAA	AAATATATAT	GCATACCTGG	ATTCCCTTG	2078
CTTCGTGAAT	GTAAGGAAA	TTAACATCTGA	ATAAATAATT	CTTCTGTGA	2128
ACTGGCTCAT	TTCTCTCTA	TGCACTGAGC	ATCTGCTCTG	TGGAAGGCC	2178
AGGATTAGTA	GTGGAGATAC	TAGGGTAAGC	CAGACACACA	CCTACCGATA	2228
GGGTATTAAG	AGTCTAGGAG	CGCGGTCTATA	TAATTAAGGT	GACAAGATGT	2278
CCTCTAACAGAT	GTAGGGAAA	AGTAACGAGT	GTGGGTATGG	GGCTCCAGGT	2328
GAGAGTGGTC	GGGTGTAAAT	TCCCTGTGTG	GGGCCTTTTG	GGCTTTGGGA	2378
AACTGCATT	TCTTCTGAGG	GATCTGATTTC	TAATGAAGCT	TGGTGGGTCC	2428
AGGGCCAGAT	TCTCAGAGGG	AGAGGGAAAA	GCCCCAGATTG	GAAAAGTTGC	2478

TCTGAGCAGT TCCTTTGTGA CAATGGATGA ACAGAGAGGA GCCTCTACCT	2528
GGG	2531

## (2) INFORMATION FOR SEQUENCE ID NO: 14:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2531 base pairs  
 (B) TYPE: nucleic acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-41 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGATCCAGGC	CCTGCCTGGA	GAAATGTGAG	GGCCCTGAGT	GAACACAGTG	50
GGGATCATCC	ACTCCATGAG	AGTGGGGACC	TCACAGAGTC	CAGCCTACCC	100
TCTTGATGGC	ACTGAGGGAC	CGGGGCTGTG	CTTACAGTCT	GCACCCCTAAG	150
GGCCCATGGA	TTCCTCTCCT	AGGAGCTCCA	GGAAACAAGGC	AGTGAGGCCT	200
TGGTCTGAGA	CAGTGTCCCTC	AGGTTACAGA	GCAGAGGATG	CACAGGCTGT	250
GCCAGCAGTG	AATGTTTGCC	CTGAATGCAC	ACCAAGGGCC	CCACCTGCCA	300
CAAGACACAT	AGGACTCCAA	AGAGTCTGGC	CTCACCTCCC	TACCATCAAT	350
CCTGCAGAAT	CGACCTCTGC	TGGCCGGCTA	TACCCCTGAGG	TGCTCTCTCA	400
CTTCCCTCTT	CAGGTTCTGA	GCAGACAGGC	CAACCGGAGA	CAGGATTCCC	450
TGGAGGCCAC	AGAGGAGCAC	CAAGGAGAAAG	ATCTGTAAGT	AAACCTTTGT	500
TAGAGCCTCT	AAGATTGGT	TCTCAGCTGA	GGTCTCTCAC	ATGCTCCCTC	550
TCTCCGTAGG	CCTGTGGTC	CCCATTGCC	AGCTTTGCC	TGCACTCTTG	600
CCTGCTGCC	TGAGCAGAGT	CATC			624
ATG TCT TCT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA					666
GGC GTT GAG GCC CAA GAA GAG GCC CTG GGC CTG GTG GGT GCG					708
CAG GCT CCT ACT ACT GAG GAG CAG GAG GCT GCT GTC TCC TCC					750
TCC TCT CCT CTG GTC CCT GGC ACC CTG GAG GAA GTG CCT GCT					792
GCT GAG TCA GCA GGT CCT CCC CAG AGT CCT CAG GGA GCC TCT					834
GCC TTA CCC ACT ACC ATC AGC TTC ACT TGC TGG AGG CAA CCC					876
AAT GAG GGT TCC AGC AGC CAA GAA GAG GAG GGG CCA AGC ACC					918
TCG CCT GAC GCA GAG TCC TTG TTC CGA GAA GCA CTC AGT AAC					960
AAG GTG GAT GAG TTG GCT CAT TTT CTG CTC CGC AAG TAT CGA					1002
GCC AAG GAG CTG GTC ACA AAG GCA GAA ATG CTG GAG AGA GTC					1044
ATC AAA AAT TAC AAG CGC TGC TTT CCT GTG ATC TTC GGC AAA					1086
GCC TCC GAG TCC CTG AAG ATG ATC TTT GGC ATT GAC GTG AAG					1128
GAA GTG GAC CCC ACC AGC AAC ACC TAC ACC CTT GTC ACC TGC					1170
CTG GGC CTT TCC TAT GAT GGC CTG CTG GGT AAT AAT CAG ATC					1212
TTT CCC AAG ACA GGC CTT CTG ATA ATC GTC CTG GGC ACA ATT					1254
GCA ATG GAG GGC GAC AGC GGC TCT GAG GAG GAA ATC TGG GAG					1296
GAG CTG GGT GTG ATG GGG GTG TAT GAT GGG AGG GAG CAC ACT					1338
GTC TAT GGG GAG CCC AGG AAA CTG CTC ACC CAA GAT TGG GTG					1380
CAG GAA AAC TAC CTG GAG TAC CGG CAG GTA CCC GGC AGT AAT					1422
CCT CGC TAT GAG TTC CTG TGG GGT CCA AGG GCT CTG GCT					1464
GAA ACC AGC TAT GTG AAA GTC CTG GAG CAT GTG GTC AGG GTC					1506
AAT GCA AGA GTT CGC ATT GCC TAC CCA TCC CTG CGT GAA GCA					1548
GCT TTG TTA GAG GAG GAA GAG GGA GTC TGA					1578
GCATGAGTGTG CAGCCAGGGC TGTGGGAAG GGGCAGGGCT GGGCCAGTGC					1628
ATCTAACAGC CCTGTGAGC AGCTTCCCT GCCTCGTGT ACATGAGGCC					1678
CATTCTTCAC TCTGTTGAA GAAAATAGTC AGTGTCTTA GTAGTGGGTT					1728
TCTATTTGTG TGGATGACTT GGAGATTATCT CTCTGTTCC TTTTACAATT					1778
GTTGAAATGT CCCTTTAAT GGATGGTTGA ATTAACCTCA GCATCCAAGT					1828
TTATGAATCG TAGTTAACGT ATATTGCTGT TAATATAGTT TAGGAGTAAG					1878
AGTCTTGTTT TTTATTCAAGA TTGGGAAATC CGTTCTATTG TGTGAATTG					1928
GGACATAATA ACAGCAGTGG AGTAAGTATT TAGAAGTGTG AATTACCGT					1978
GAAATAGGTG AGATAAATTAA AAAGATACTT AATTCCCGCC TTATGCCTCA					2028
GTCTATTCTG TAAAATTAA AAATATATAT GCATACCTGG ATTTCCCTGG					2078
CTTCGTGAAT GTAAGAGAAA TTAAATCTGA ATAAAATAATT CTTCTGTAA					2128
ACTGGCTCAT TTCTCTCTA TGCACGTGAGC ATCTGCTCTG TGGAAAGGCC					2178
AGGATTAGTA GTGGAGATAC TAGGGTAAGC CAGACACACA CCTACCGATA					2228

GGGTATTAAG	AGTCTAGGAG	CGCGGTCTATA	TAATTAAGGT	GACAAGATGT	2278
CCTCTAAGAT	GTAGGGAAA	AGTAACGAGT	GTGGGTATGG	GGCTCCAGGT	2328
GAGAGTGGTC	GGGTGTAAT	TCCCTGTGTG	GGGCCTTTG	GGCTTTGGGA	2378
AACTCCATT	TCTTCTGAGG	GATCTGATT	TAATGAAGCT	TGGTGGGTCC	2428
AGGGCCAGAT	TCTCAGAGGG	AGAGGGAAA	GCCCAGATTG	GAAAAGTTGC	2478
TCTGAGCGGT	TCCTTGTGA	CAATGGATGA	ACAGAGAGGA	GCCTCTACCT	2528
GGG					2531

## (2) INFORMATION FOR SEQUENCE ID NO: 15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1068 base pairs
  - (B) TYPE: nucleic acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA
- (ix) FEATURE:
  - (A) NAME/KEY: cDNA MAGE-4
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

G	GGG	CCA	AGC	ACC	TCG	CCT	GAC	GCA	GAG	TCC	TTG	TTC	CGA	40
GAA	GCA	CTC	AGT	AAC	AAG	G TG	GAT	GAG	TTG	GCT	CAT	TTT	CTG	82
CTC	CGC	AAG	TAT	CGA	GCC	AAG	GAG	CTG	GTC	ACA	AAG	GCA	GAA	124
ATG	CTG	GAG	AGA	GTC	ATC	AAA	AAT	TAC	AAG	CGC	TGC	TTT	CCT	166
GTG	ATC	TTC	GGC	AAA	GCC	TCC	GAG	TCC	CTG	AAG	ATG	ATC	TTT	208
GGC	ATT	GAC	GTG	AAG	GAA	G TG	GAC	CCC	GCC	AGC	AAC	ACC	TAC	250
ACC	CTT	GTC	ACC	TGC	CTG	GGC	CTT	TCC	TAT	GAT	GGC	CTG	CTG	292
GGT	AAT	AAT	CAG	ATC	TTT	CCC	AAG	ACA	GGC	CTT	CTG	ATA	ATC	334
GTC	CTG	GGC	ACA	ATT	GCA	ATG	GAG	GGC	GAC	AGC	GCC	TCT	GAG	376
GAG	GAA	ATC	TGG	GAG	GAG	CTG	GGT	GTG	ATG	GGG	GTG	TAT	GAT	418
GGG	AGG	GAG	CAC	ACT	GTC	TAT	GGG	GAG	CCC	AGG	AAA	CTG	CTC	460
ACC	CAA	GAT	TGG	GTG	CAG	GAA	AAC	TAC	CTG	GAG	TAC	CCG	CAG	502
GTA	CCC	GGC	AGT	ATT	CCT	CGC	CGC	TAT	GAG	TTC	CTG	TGG	GGT	544
CCA	AGG	GCT	CTG	GCT	GAA	ACC	AGC	TAT	GTG	AAA	GTC	CTG	GAG	586
CAT	GTG	GTC	AGG	GTC	AAT	GCA	AGA	GTT	CGC	ATT	GCC	TAC	CCA	628
TCC	CTG	CGT	GAA	GCA	GCT	TTG	TTA	GAG	GAG	GAA	GAG	GGA	GTC	670
TGAGCATGAG	TTGCAGCCAG	GGCTGTGGGG	AAGGGCCAGG	GCTGGGCCAG										720
TGCATCTAAC	AGCCCTGTGC	AGCAGCTTCC	CTTGCTCGT	GTAACATGAG										770
GCCCCATTCTT	CACTCTGTTT	GAAGAAAATA	GTCAGTGTTC	TTAGTAGTGG										820
GTTTCTATT	TGTTGGATGA	CTTGGAGATT	TATCTCTGTT	TCCCTTTACA										870
ATTGTTGAAA	TGTTCTTTT	AATGGATGGT	TGAATTAACT	TCAGCATCCA										920
AGTTTATGAA	TCGTAGTTAA	CGTATATTGC	TGTTAAATATA	GTTTAGGAGT										970
AAGAGTCTTG	TTTTTTATTC	AGATTGGGAA	ATCCGTTCTA	TTTGTGAAT										1020
TTGGGACATA	ATAACAGCAG	TGGAGTAAGT	ATTTAGAAGT	GTGAATT										1068

## (2) INFORMATION FOR SEQUENCE ID NO: 16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2226 base pairs
  - (B) TYPE: nucleic acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (ix) FEATURE:
  - (A) NAME/KEY: MAGE-5 gene
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GGATCCAGGC	CTTGCCAGGA	GAAAGGTGAG	GGCCCTGTGT	GAGCACAGAG	50
GGGACCATTC	ACCCCCAAGAG	GGTGGAGACC	TCACAGATT	CAGCCTACCC	100
TCCCTGTTAGC	ACTGGGGGCC	TGAGGCTGTG	CTTGCAGTCT	GCACCCCTGAG	150
GGCCCCATGCA	TTCCCTCTTCC	AGGAGCTCCA	GGAAACAGAC	ACTGAGGCCT	200
TGGTCTGAGG	CCGTGCCCTC	AGGTCACAGA	GCAGAGGAGA	TGCAGACGTC	250
TAGTGCCAGC	AGTGAACGTT	TGCGCTTGAAT	GCACACTAAT	GGCCCCCATC	300

GCCCCAGAAC	ATATGGGACT	CCAGAGCACC	TGGCCTCACC	CTCTCTACTG	350
TCAGTCCTGC	AGAATCAGCC	TCTGCTTGCT	TGTGTACCCCT	GAGGTGCCCT	400
CTCACTTTT	CCTTCAGGTT	CTCAGGGGAC	AGGCTGACCA	GGATCACCA	450
GAAGCTCCAG	AGGATCCCCA	GGAGGCCCTA	GAGGAGCACC	AAAGGAGAAG	500
ATCTGTAAGT	AAGCCTTGT	TAGAGCCTCC	AAGGTTCACT	TTTTAGCTGA	550
GGCTTCTCAC	ATGCTCCCTC	TCTCTCCAGG	CCAGTGGGTC	TCCATTGCC	600
AGCTCCTGCC	CACACTCTG	CCTGTTGCCG	TGACCAAGAGT	CGTC	644
ATG TCT CTT	GAG CAG AAG	AGT CAG CAC	TGC AAG CTC	GAG GAA	684
CTC CTC TGG	TCC CAG GCA	CCC TGG GGG	AGG TGC CTG	CTG CTG CTG	728
GGT CAC CAG	GTC CTC TCA	AGA GTC CTC	ACG GAG CCT	CCG CCA	770
TCC CCA CTG	CCA TCG ATT	TCA CTC TAT	GGA GGC AAT	CCA TTA	812
AGG GCT CCA	GCA ACC AAG	AGG AGG GGC	CAA GCA CCT	CCC	854
CTG ACC CAG	AGT CTG TGT	TCC GAG CAG	CAC TCA GTA	AGA AGG	896
TGG CTG ACT	TGA				908
TTCATTTCT	GCTCTCAAG	TATTAAGTCA	AGGAGCTGGT	CACAAAGGCA	958
GAAATGCTGG	AGACCGTCAT	CAAAAATTAC	AAGCGCTGCT	TTCCCTGAGAT	1008
CTTCGGCAAA	GCCTCCGAGT	CCTTCAGCT	GGTCTTTGGC	ATTGACGTGA	1058
AGGAAGCGGA	CCCCACCCAGC	AAACACCTACA	CCCTTGTAC	CTGCCCTGGGA	1108
CTCCTATGAT	GGCCTGCTGG	TTGATAATAA	TCAGATCATG	CCCAAGACGG	1158
GCCTCCTGAT	AATCGTCTTG	GGCATGATTG	CAATGGAGGG	CAAATGCGTC	1208
CCTGAGGAGA	AAATCTGGGA	GGAGCTGAGT	GTGATGAAGG	TGTATGTTGG	1258
GAGGGAGCAC	AGTGTCTGTG	GGGAGCCAG	GAAGCTGCTC	ACCCAAGATT	1308
TGGTGCAGGA	AAACTACCTG	GAGTACCGGC	ACGTGCCAG	CAGTGATCCC	1358
ATATGCTATG	AGTTACTGTG	GGGTCCAAGG	GCACTCGCTG	CTTGAAGTA	1408
CTGGAGCAGC	TGGTCAGGGT	CAATGCAAGA	GTTCTCATTT	CTTACCCATC	1458
CCTCGCTGAA	GCACCTTTGA	GAGAGGAGGA	AGAGGGAGTC	TGAGCATGAG	1508
CTGCAGCCAG	GGCCACTGCG	AGGGGGCTG	GGCCAGTGCA	CCTTCCAGGG	1558
CTCCGTCCAG	TAGTTTCCCC	TGCCTTAATG	TGACATGAGG	CCCATTCTTC	1608
TCTCTTTGAA	GAGAGCAGTC	AACATTCTTA	GTAGTGGGTT	TCTGTTCTAT	1658
TGGATGACTT	TGAGATTGT	CTTTGTTTCC	TTTTGGAATT	GTTCAAATGT	1708
TTCTTTTAAT	GGGTGGTTGA	ATGAACTICA	GCATTCAAAT	TTATGAATGA	1758
CAGTACTCAC	ACATAGTGCT	GTTTATATAG	TTTACGGAGTA	AGAGTCTTGT	1808
TTTTTATTCA	GATTGGAAA	TCCATTCCAT	TTTGTGAATT	GGGACATAGT	1858
TACAGCAGT	GAATAAGTAT	TCATTTAGAA	ATGTAATGA	GCAGTAAAAC	1908
TGATGACATA	AAAGAATTAA	AAAGATATTAA	ATTCTTGCTT	ATACTCAGTC	1958
TATTCCGTAA	AATTTTTTTT	AAAAAAATGTG	CATACCTGGA	TTTCCTTGGC	2008
TTCTTTGAGA	ATGTAAGACA	AATTAAATCT	GAATAAATCA	TTCTCCCTGT	2058
TCACTGGCTC	ATTTATTCTC	TATGCACTGA	GCATTGCTC	TGTGGAAGGC	2108
CCTGGGTAA	TAGTGGAGAT	GCTAAGGTAA	GCCAGACTCA	CCCCTACCCA	2158
CAGGGTAGTA	AACTCTAGGA	GCAGCAGTCA	TATAATTAAG	GTGGAGAGAT	2208
GCCCTCTAAG	ATGTAGAG				2226

## (2) INFORMATION FOR SEQUENCE ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2305 base pairs
  - (B) TYPE: nucleic acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (ix) FEATURE:
  - (A) NAME/KEY: MAGE-51 gene
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GGATCCAGGC	CTTGCAGGA	AAAAGGTAG	GGCCCTGTGT	GAGCACAGAG	50
GGGACCATTC	ACCCCAAGAG	GGTGGAGACC	TCACAGATTG	CAGCCTACCC	100
TCCTGTTAGC	ACTGGGGGCC	TGAGGCTGTG	CTTGCAGTCT	GCACCCCTGAG	150
GGCCCATGCA	TTCCCTTCTC	AGGAGCTCCA	GGAAACAGAC	ACTGAGGCCT	200
TGGTCTGAGG	CCGTGCCCTC	AGGTACACAGA	GCAGAGGAGA	TGCAGACGTC	250
TAGTGCCAGC	AGTGAACGTT	TGCCCTGAAT	GCACACTAAT	GGCCCCCATC	300
GCCCCAGAAC	ATATGGGACT	CCAGAGCACC	TGGCCTCACC	CTCTCTACTG	350
TCAGTCCTGC	AGAATCAGCC	TCTGCTTGCT	TGTGTACCCCT	GAGGTGCCCT	400
CTCACTTTT	CCTTCAGGTT	CTCAGGGGAC	AGGCTGACCA	GGATCACCA	450
GAAGCTCCAG	AGGATCCCCA	GGAGGCCCTA	GAGGAGCACC	AAAGGAGAAG	500
ATCTGTAAGT	AAGCCTTGT	TAGAGCCTCC	AAGGTTCACT	TTTTAGCTGA	550

GGCTTCTCAC	ATGCTCCCTC	TCTCTCCAGG	CCAGTGGGT	TCCATTGCC	600
AGCTCCTGCC	CACACTCCTG	CCTGTTGCCG	TGACCAAGT	CGTC	644
ATG TCT CTT	GAG CAG AAG AGT	CAG CAC TGC	AAG CCT GAG	GAA	686
GGC CTT GAC	ACC CAA GAA GAG	CCC TGG GCC	TGG TGC	TGC	728
AGG CTG CCA	CTA CTG AGG AGC	AGG AGG CTG	TGT CCT CCT	CCT	770
CTC CTC TGG	TCC CAG GCA CCC	TGG GGG AGG	TGC AGG GAG	TGC	812
GGT CAC CAG	GTC CTC TCA AGA	GTC CTC AGG	GAG CCT CCG	CCA	854
TCC CCA CTG	CCA TCG ATT TCA	CTC TAT GGA	GGA AAT CCA	TTA	896
AGG GCT CCA	GCA ACC AAG AGG	GGG CAA GCA	CCT GCA	CCC	938
CTG ACC CAG	AGT CTG TGT TCC	GAG CAC TCA	GTA AGA AGG		980
TGG CTG ACT	TGA				992
TTCATTTCT	GCTCCTCAAG	TATTAAGTCA	AGGAGCCGGT	CACAAAGGCA	1042
GAATGCTGG	AGAGCGTCAT	AAAAAATTAC	AAGCGCTGCT	TTCCCTGAGAT	1092
CTTCGGCAAA	GCCTCCGAGT	CCTTGCAGCT	GGTCTTGGC	ATTGACGTGA	1142
AGGAAGCCGA	CCCCACCCAGC	AACACCTACA	CCCTTGTCA	CTGCCTGGGA	1192
CTCTATGAT	GGCCTGGTGG	TTAACATAGA	TCATGCCAA	GACGGGCCTC	1242
CTGATAATCG	TCTTGGGATC	GATTGCAATG	GAGGGCAAAT	GGCTCCCTGA	1292
GGAGAAAATC	TGGGAGGAGC	GGGGTGTGAT	GAAGGTGTAT	GTTGGAGGG	1342
AGCACAGTGT	CTGTGGGGAG	CCCAGGAAGC	TGTCACCCCA	AGATTTGGTG	1392
CAGGAAAATC	ACCTGGAGTA	CCGCAGGTGC	CCAGCAGTGA	TCCCCATATGC	1442
TATGAGTTAC	TGTGGGGTCC	AAGGGCACTC	GCTGCTTGAA	AGTACTGGAG	1492
CACGTGGTCA	GGGTCAATGC	AAGAGTTCTC	ATTTCTTAC	CATCCCCTGCA	1542
TGAAGCAGCT	TTGAGAGAGG	AGGAAGAGGG	AGTCTGAGCA	TGAGCTGCAG	1592
CCAGGGGCCAC	TGGCAGGGGG	GCTGGGCCAG	TGCACCTTCC	AGGGCTCCGT	1642
CCAGTAGTTT	CCCCCTGCCT	AATGTGACAT	GAGGCCATT	CTTCTCTCTT	1692
TGAAGAGAGC	AGTCAACATT	CTTAGTAGTG	GGTTTCTGTT	CTATGGATG	1742
ACTTTGAGAT	TTGCTTTG	TTCTCTTGG	AATTGTTCAA	ATGTTCTTT	1792
TAATGGGTGG	TTGAATGAAC	TTCACTTATC	AAATTATGAA	ATGACAGTAG	1842
TCACACATAG	TGCTGTTTAT	ATAGTTAGG	AGTAAGAGTC	TTGTTTTTTA	1892
TTCAAGATGG	GAATCCATT	CCATTTGTG	AATTGGACA	TAGTTACAGC	1942
AGTGGAAATA	GTATTCACTT	AGAAATGTGA	ATGAGCAGTA	AAACTGATGA	1992
GATAAAGAAA	TTAAAAGATA	TTTAATTCTT	GCCTTATACT	CAGTCTATT	2042
GGTAAAATTT	TTTTTTAAAA	ATGTGCATAC	CTGGATTTC	TTGGCTCTT	2092
TGAGAATGTA	AGACAAATT	AATCTGAATA	AATCATTCTC	CCTGTTCACT	2142
GGCTCATTTA	TTCTCTATGC	ACTGAGCATT	TGCTCTGTGG	AAGGCCCTGG	2192
GTAAATAGTG	GAGATGCTAA	GGTAAGCAG	ACTCACCCCT	ACCCACAGGG	2242
TAGTAAAGTC	TAGGAGCAGC	AGTCATATAA	TTAAGGTGGA	GAGATGCCCT	2292
CTAAGATGTA	GAG				2305

(2) INFORMATION FOR SEQUENCE ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 225 base pairs
- (B) TYPE: nucleic acid
- (C) TOPOLOGY: linear

(B) TOPOLOGY: Line  
(iii) MOLECULE TYPE: —DNA

(ii) MOLECULE  
(iv) FEATURES

(ix) FEATURE: (A) NAME/KEY: MAGE-6 2000

(A) NAME/KEY: MAGE-6 gene  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18.

## (2) INFORMATION FOR SEQUENCE ID NO: 19:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1947 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: MAGE-7 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TGAATGGACA	ACAAGGGCCC	CACACTCCCC	AGAACACAAG	GGACTCCAGA	50
GAGCCCAGCC	TCACCTTCCC	TACTGTCAGT	CCTGAGCCT	CAGCCCTCTGC	100
TGGCCGGCTG	TACCCCTGAGG	TCCCCTCTCA	CTTCCTCCCT	CAGGTTCTCA	150
GCGGCACAGGC	CGGCCAGGAG	GTCAGAAGCC	CCAGGAGGCC	CCAGAGGAGC	200
ACCGAACAGGAG	AAGATCTGTA	AGTAGGCTCT	TGTTAGGGCC	TCCAGGGCGT	250
GGTTTACAAA	TGAGGCCCCCT	CACAAGCTCC	TTCTCTCCCC	AGATCTGTGG	300
GTTCCTCCCC	ATCGCCCAGC	TGCTGCCCGC	ACTCCAGCCT	GCTGCCCTGA	350
CCAGAGTCAT	CATGTCTTCT	GAGCAGAGGA	GTCAGCACTG	CAAGCCTGAG	400
GATGCCCTGA	GGCCAAGGA	CAGGAGGCTC	TGGGCTGGT	GGGTGCGCAG	450
GCTCCCGCCA	CCGAGGAGCA	CGAGGCTGCC	TCCTCCTTCA	CTCTGATTGA	500
AGGCACCCCTG	GAGGAGGTGC	CTGCTGCTGG	GTCCCCCAGT	CCTCCCTCTGA	550
GTCTCAGGGT	TCCTCCTTTT	CCCTGACCAT	CAGCAACAAAC	ACTCTATGGA	600
GCCAATCCAG	TGAGGGCAC	AGCAGCCGGG	AAGAGGAGGG	GCCAACCACC	650
TAGACACACC	CCGCTCACCT	GGCGTCCTTG	TTCCA		685
ATG GGA AGG	TGG CTG AGT	TGG TTC GCT	TCC TGC TGC	ACA AGT	727
ATC GAG TCA	AGG AGC TGG	TCA CAA AGG	CAG AAA TGC	TGG ACA	769
GTG TCA TCA	AAA ATT ACA	AGC ACT AGT	TTC CTT GTG	ATC TAT	811
GGC AAA GCC	TCA GAG TGC	ATG CAG GTG	ATG TTT GGC	ATT GAC	853
ATG AAG GAA	GTG GAC CCC	GCG ACT CCT	ACG TCC TTG	TCA	895
CCT GCT TGG	GCC TCT ACA	ATG GCC TGG	GTG ATG ATC		937
AGA GCA TGC	CCG AGA CGG	TTC GCC TGA			964
TTATGGCTTT	GACCATGATC	TTAATGGAGG	GCCACTGTGC	CCCTGAGGAG	1014
GCAATCTGGG	AAGCGTTGAG	TGTAATGGTG	TATGATGGGA	TGGAGCAGTT	1064
TCTTGGGCA	GCTGAGGAAG	CTGCTCACCC	AAGATTGGGT	GCAGGAAAAC	1114
TACCTGCAAT	ACCGCCAGGT	GCCCAGCAGT	GATCCCCCGT	GCTACCAGTT	1164
CCTGTGGGGT	CCAAGGGCCC	TCATTGAAAC	CAGCTATGTG	AAAGTCTCTGG	1214
AGTATGCAAC	CAGGGTCAGT	ACTAAAGAGA	GCATTCCTCA	CCCATCCCTG	1264
CATGAAGAGG	CTTTGGGAGA	GGAGGAAGAG	GGAGTCTGAG	CAGAAGTTGC	1314
AGCCAGGGCC	AGTGGGGCAG	ATTGGGGAG	GGCCCTGGCA	GTGCACGTT	1364
CACACATCCA	CCACCTTCCC	TGTCCTGTA	CATGAGGCC	ATTCTTCACT	1414
CTGTGTTTGA	AGAGAGCAGT	CAATGTTCTC	ACTAGCGGGG	AGTGTGTTGG	1464
GTGTGAGGGG	ATACAAGGTG	GACCATCTCT	CAGTCCCTGT	TCTCTTGGGC	1514
GATTTGGAGG	TTTATCTTTG	TTTCCTTTG	CAGTCGTTCA	AATGTTCTT	1564
TTAATGGATG	GTGTAATGAA	CTTCAACATT	CATTCTCATGT	ATGACAGTAG	1614
GCAGACTTAC	TGTTTTTTAT	ATAGTTAAA	CTAAGTGCAT	TGTTTTTTAT	1664
TTATGTAAGA	AAATCTATGT	TATTTCTGTA	ATTGGGACAA	CATAACATAG	1714
CAGAGGATTA	AGTACCTTTT	ATAATGTGAA	AGAACAAAGC	GGTAAAATGG	1764
GTGAGATAAA	GAATAAAAGA	AATTAATATG	GCTGGGCACG	GTGGCTCACG	1814
CCTGTAATCC	CAGCACTTTA	GGAGGCAGAG	GCACGGGGAT	CACGAGGTCA	1864
GGAGATCGAG	ACCATTCTGG	CTAACACAGT	GAAACACCAT	CTCTATTAAA	1914
AATACAAAAC	TTAGCCGGGC	GTGGTGGCGG	GTG		1947

## (2) INFORMATION FOR SEQUENCE ID NO: 20:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1810 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: MAGE-8 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GAGCTCCAGG	AACCAGGCTG	TGAGGTCTTG	GTCTGAGGCA	GTATCTCAA	50
TCACAGAGCA	TAAGAGGCC	AGGCAGTAGT	AGCAGTCAAG	CTGAGGTGGT	100

GTTCCTCCCTG	TATGTATACC	AGAGGCCCC	CTGGCATCAG	AACAGCAGGA	150
ACCCACAGT	TCCTGGCCCT	ACCAGCCCTT	TTGTCAGTCC	TGGAGCCTTG	200
GCCTTGCCA	GGAGGCTGCA	CCCTGAGATG	CCCTCTCAAT	TTCTCCTTCA	250
GGTCGCAGA	GAACAGGCCA	GCCAGGAGGT	CAGGAGGCC	CAGAGAAGCA	300
CTGAAGAAGA	CCTGTAAGTA	GACCTTTGTT	AGGGCATCCA	GGGTGTTAGTA	350
CCCAGCTGAG	GCCTCTCAC	CGCTTCCCTCT	CTCCCCAGGC	CTGTGGGTCT	400
CAATTGCCCA	GCTCCGGCCC	ACACTCTCCT	GCTGCCCTGA	CCTGAGTCAT	450
C					451
ATG CTT CTT GGG CAG AAG AGT CAG CGC TAC AAG GCT GAG GAA	493				
GCG CTT CAG GCC CAA GGA GAG GCA CCA GGG CTT ATG GAT GTG	535				
CAG ATT CCC ACA GCT GAG GAG CAG AAG GCT GCA TCC TCC TCC	577				
TCT ACT CTG ATC ATG GGA ACC CTT GAG GAG GTG ACT GAT TCT	619				
GGG TCA CCA AGT CCT CCC CAG AGT CCT GAG GGT GCC TCC TCT	661				
TCC CTG ACT GTC ACC GAC AGC ACT CTG TGG AGC CAA TCC GAT	703				
GAG GGT TCC AGC AGC AAT GAA GAG GAG GGG CCA AGC ACC TCC	745				
CCG GAC CCA GCT CAC CTG GAG TCC CTG TTC CGG GAA GCA CTT	787				
GAT GAG AAA GTG GCT GAG TTA GTT CGT TTC CTG CTC CGC AAA	829				
TAT CAA ATT AAG GAG CCG GTC ACA AAG GCA GAA ATG CTT GAG	871				
AGT GTC ATC AAA AAT TAC AAG AAC CAC TTT CCT GAT ATC TTC	913				
AGC AAA GCC TCT GAG TGC ATG CAG GTG ATC TTT GGC ATT GAT	955				
GTG AAG GAA GTG GAC CCT GCC GGC CAC TCC TAC ATC CTT GTC	997				
ACC TGC CTG GGC CTC TCC TAT GAT GGC CTG CTG GGT GAT GAT	1039				
CAG AGT ACG CCC AAG ACC GGC CTC CTG ATA ATC GTC CTG GGC	1081				
ATG ATC TTA ATG GAG GGC AGC CGC GCC CCG GAG GAG GCA ATC	1123				
TGG GAA GCA TTG AGT GTG ATG GGG GCT GTA TGA	1156				
TGGGAGGGAG CACAGTGTCT ATTGGAAGCT CAGGAAGCTG CTCACCCAAAG	1206				
AGTGGGTGCA GGAGAACTAC CTGGAGTACCC GCCAGGCGCC CGGCAGTGAT	1256				
CCTGTGCGCT ACGAGTTCT GTGGGGTCCA AGGGCCCTTG CTGAAACCCAG	1306				
CTATGTGAAA GTCTGGAGC ATGTGGTCAG GGTCAATGCA AGAGTTCGCA	1356				
TTTCTTACCC ATCCCTGCAT GAAGAGGCTT TGGGAGAGGA GAAAGGAGTT	1406				
TGAGCAGGAG TTGCAGCTAG GGCCAGTGGG GCAGGTTGTG GGAGGGCCTG	1456				
GGCCAGTGCACGTTCCAGGG CCACATCCAC CACTTCCCT GCTCTGTTAC	1506				
ATGAGGCCCA TTCTTCACTC TGTGTTGAA GAGAGCAGTC ACAGTTCTCA	1556				
GTAGTGGGGAC GCATGTTGGG TGTGAGGGAA CACAGTGTGG ACCATCTCTC	1606				
AGTTCTGTGTT CTATGGGGCG ATTTGGAGGT TTATCTTTGT TTCCTTTGG	1656				
AATTGTTCCA ATGTTCTTC TAATGGATGG TGTAAATGAAAC TTCAACATTC	1706				
ATTTTATGTA TGACAGTAGA CAGACTTACT GCTTTTATA TAGTTTAGGA	1756				
GTAAGAGTCT TGCTTTCAT TTATACTGGG AAACCCATGT TATTTCTTGA	1806				
ATTC	1810				

## (2) INFORMATION FOR SEQUENCE ID NO: 21:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1412 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: genomic DNA

## (ix) FEATURE:

- (A) NAME/KEY: MAGE-9 gene

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TCTGAGACAG	TGTCTCAGG	TCGCAGAGCA	GAGGAGACCC	AGGCAGTGTC	50
ACCAAGTGAAG	GTGAAGTGT	CACCCCTGAAT	GTGCAACCAAG	GGCCCCACCT	100
GCCCCAGCAC	ACATGGGACC	CCATAGCACC	TGGCCCCATT	CCCCCTACTG	150
TCACTCATAG	AGCCTTGATC	TCTGCAGGCT	AGCTGCACGC	TGAGTAGCCC	200
TCTCACTTCC	TCCCTCAGGT	TCTCGGGACA	GGCTAACCG	GAGGACAGGA	250
GCCCCAAGAG	GCCCCAGAGC	AGCACTGACC	AAGACCTGTA	AGTCAGCCTT	300
TGTTAGAAC	TCCAAGGTT	GGTTCTCAGC	TGAAGTCTCT	CACACACTCC	350
CTCTCTCCCC	AGGCCTGTGG	GTCTCCATCG	CCCAGCTCCT	GCCCCACGCTC	400
CTGACTGCTG	CCCTGACCAAG	AGTCATC			427
ATG TCT CTC GAG CAG AGG AGT CCG CAC TGC AAG CCT GAT GAA	469				
GAC CTT GAA GCC CAA GGA GAG GAC TTG GGC CTG ATG GGT GCA	511				
CAG GAA CCC ACA GGC GAG GAG GAG GAG ACT ACC TCC TCC TCT	553				
GAC AGC AAG GAG GAG GTG TCT GCT GCT GGG TCA TCA AGT	595				

CCT	CCC	CAG	AGT	CCT	CAG	GGA	GGC	GCT	TCC	TCC	TCC	ATT	TCC	637
GTC	TAC	TAC	ACT	TTA	TGG	AGC	CAA	TTC	GAT	GAG	GGC	TCC	AGC	679
AGT	CAA	GAA	GAG	GAA	GAG	CCA	AGC	TCC	TGG	GTC	GAC	CCA	GCT	721
CAG	CTG	GAG	TTC	ATG	TTC	CAA	GAA	GCA	CTG	AAA	TTG	AAG	GTG	763
GCT	GAG	TTG	GTT	CAT	TTC	CTG	CTC	CAC	AAA	TAT	CGA	GTC	AAG	805
GAG	CCG	GTC	ACA	AAG	GCA	GAA	ATG	CTG	GAG	AGC	GTC	ATC	AAA	847
AAT	TAC	AAG	CGC	TAC	TTT	CCT	GTG	ATC	TTC	GGC	AAA	GCC	TCC	889
GAG	TTC	ATG	CAG	GTG	ATC	TTT	GGC	ACT	GAT	GTG	AAG	GAG	GTG	931
GAC	CCC	GCC	GGC	CAC	TCC	TAC	ATC	CTT	GTC	ACT	GCT	CTT	GGC	973
CTC	TCG	TGC	GAT	AGC	ATG	CTG	GGT	GAT	GGT	CAT	AGC	ATG	CCC	1015
AAG	GCC	GCC	CTC	CTG	ATC	ATT	GTC	CTG	GGT	GTG	ATC	CTA	ACC	1057
AAA	GAC	AAC	TGC	GCC	CCT	GAA	GAG	GTT	ATC	TGG	GAA	GCG	TTG	1099
AGT	GTG	ATG	GGG	GTG	TAT	GTT	GGG	AAG	GAG	CAC	ATG	TTC	TAC	1141
GGG	GAG	CCC	AGG	AAG	CTG	CTC	ACC	CAA	GAT	TGG	GTG	CAG	GAA	1183
AAC	TAC	CTG	GAG	TAC	CGG	CAG	GTG	CCC	GGC	AGT	GAT	CCT	GCG	1225
CAC	TAC	GAG	TTC	CTG	TGG	GGT	TCC	AAG	GCC	CAC	GCT	GAA	ACC	1267
AGC	TAT	GAG	AAG	GTC	ATA	AAT	TAT	TTG	GTC	ATG	CTC	AAT	GCA	1309
AGA	GAG	CCC	ATC	TGC	TAC	CCA	TCC	CTT	TAT	GAA	GAG	GTT	TTG	1351
GGA	GAG	GAG	CAA	GAG	GGA	GTC	TGA							1375
GCACCAAGCCG CAGCCGGGG CAAAGTTGT GGGGTCA														1412

## (2) INFORMATION FOR SEQUENCE ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 920 base pairs
  - (B) TYPE: nucleic acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (ix) FEATURE:
  - (A) NAME/KEY: MAGE-10 gene
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

ACCTGCTCCA	GGACAAAGTG	GACCCCAC	TG	CATCAGCTCC	ACCTACCC	TA	50							
CTGTCAGTCC	TGGAGCCTTG	GCCTCTGCCG	GCTGCATCCT	GAGGAGCCAT			100							
CTCTCACTTC	CTTCTTCAGG	TTCTCAGGGG	ACAGGGAGAG	CAAGAGGTCA			150							
AGAGCTGTGG	GACACCAACAG	AGCAGCACTG	AAGGAGAAGA	CCTGTAAGTT			200							
GGCC	TTTGTGTT	AGAACCTCCA	GGGTGTGGTT	CTCAGCTGTG	GCCACTTACA		250							
CCCT	CCCCTCT	CTCCCCAGGC	CTGTGGGTCC	CCATCGCCCCA	AGTCCTGCC		300							
ACACT	CCCAC	CTGCTACCC	GATCAGAGTC	ATC			333							
ATG	CCT	CGA	GCT	CCA	AAG	CGT	CAG	CGC	TGC	ATG	CCT	GAA	GAA	375
GAT	CTT	CAA	TCC	CAA	AGT	GAG	ACA	CAG	GGC	CTC	GAG	GGT	GCA	417
CAG	GCT	CCC	CTG	GCT	GTG	GAG	GAT	GCT	TCA	TCA	TCC	ACT		459
TCC	ACC	AGC	TCC	TCT	TTT	CCA	TCC	TCT	TTT	CCC	TCC	TCC	TCC	501
TCT	TCC	TCC	TCC	TCC	TCC	TGC	TAT	CCT	CTA	ATA	CCA	AGC	ACC	543
CCA	GAG	GAG	GTT	TCT	GCT	GAT	GAT	GAG	ACA	CCA	AAT	CCT	CCC	585
CAG	AGT	GCT	CAG	ATA	GCC	TGC	TCC	TCC	CCC	TCG	GTC	GTT	GCT	627
TCC	CTT	CCA	TTA	GAT	CAA	TCT	GAT	GAG	GGC	TCC	AGC	AGC	CAA	669
AAG	GAG	GAG	AGT	CCA	AGC	ACC	CTA	CAG	GTC	CTG	CCA	GAC	AGT	711
GAG	TCT	TTA	CCC	AGA	AGT	GAG	ATA	GAT	GAA	AAG	GTG	ACT	GAT	753
TTG	GTG	CAG	TTT	CTG	CTC	TTC	AAG	TAT	CAA	ATG	AAG	GAG	CCG	795
ATC	ACA	AAG	GCA	GAA	ATA	CTG	GAG	AGT	GTC	ATA	AAA	AAT	TAT	837
GAA	GAC	CAC	TTC	CCT	TTG	TTG	TTT	AGT	GAA	GCC	TCC	GAG	TGC	879
ATG	CTG	CTG	GTC	TTT	GGC	ATT	GAT	GTA	AAG	GAA	GTG	GAT	CC	920

## (2) INFORMATION FOR SEQUENCE ID NO: 23:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1107 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

## (ix) FEATURE:

- (A) NAME/KEY: MAGE-11 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

AGAGAACAGG	CCAAACCTGGA	GGACAGGGAGT	CCCAGGGAGAA	CCCAGAGGAT	50
CACTGGAGGA	GAACAAGTGT	AAAGTAGGCT	TTGTTAGATT	CTCCATGGTT	100
CATATCTCAT	CTGAGCTCTGT	TCTCACGCTC	CCTCTCTCCC	CAGGCTGTGG	150
GGCCCCATCA	CCCGAGATATT	TCCCACAGGT	CGGCCCTGCTG	ACCTAACCRAG	200
AGTCATCATG	CCTCTTGAGC	AAAGAAGTCA	GCAC TGCAAG	CCTGAGGAAG	250
CCTTCAGGGCC	CAAGAAGAAG	ACCTGGGCT	GGTGGGTGCA	CAGGCTCTCC	300
AAGCTGAGGA	GCAGGAGGCT	GCCTTCTCT	CCTCTACTCT	GAATGTGGGC	350
ACTCTAGAGG	AGTTGCCTGC	TGCTGAGTCA	CCAAGTCCTC	CCCAGAGTCC	400
TCAGGAAGAG	TCCCTCTCTC	CCACTGCCAT	GGATGCCATC	TTTGGGAGCC	450
TATCTGATGA	GGGCTCTGGC	AGCCAAGAAA	AGGAGGGGCC	AA GTACCTCG	500
CCTGACCTGA	TAGACCCCTGA	GTCCCTTTTCC	CAAGATATAAC	TACATGACAA	550
GATAATTGAT	TTGGTTCAATT	TATTCTCCGC	AA GTATCGAG	TCAAGGGGCT	600
GATCACAAAG	GCAGAGA				616
ATG CTG GGG	AGT GTC ATC	AAA AAT TAT	GAG GAC TAC	TTT CCT	658
GAG ATA TTT	AGG GAA GCC	TCT GTA TGC	ATG CAA CTG	CTC TTT	700
GGC ATT GAT	GTG AAG GAA	GTG GAC CCC	ACT AGC CAC	TCC TAT	742
GTC CTT GTC	ACC TCC CTC	AAC CTC TCT	TAT GAT GGC	ATA CAG	784
TGT AAT GAG	CAG AGC ATG	CCC AAG TCT	GGC CTC CTG	ATA ATA	826
GTC CTG GGT	GTA ATC TTC	ATG GAG GGG	AAC TGC ATC	CCT GAA	868
GAG GTT ATG	TGG GAA GTC	CTG AGC ATT	ATG GGG GTG	TAT GCT	910
GGA AGG GAG	CAC TTC CTC	TTT GGG GAG	CCC CAA AGG	AGG CTC CTT	952
ACC CAA AAT	TGG GTG CAG	GAA AAG TAC CTG	GTG TAC CGG	CAG CAG	994
G TG CCC GGC	ACT GAT CCT	GCA TGC TAT	GAG TTC CTG	TGG GGT	1036
CCA AGG GCC	CAC GCT GAG	ACC AGC AAG ATG	AAA GTT CTT	GAG GAG	1078
TAC ATA GCC	AAT GCC AAT	GGG AGG GAT	CC		1107

## (2) INFORMATION FOR SEQUENCE ID NO: 24:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2150 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

## (ix) FEATURE:

- (A) NAME/KEY: smage-I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

TCTGTCTGCA	TATGCCCTCCA	CTTGTGTGTA	GCAGTCTCAA	ATGGATCTCT	50
CTCTACAGAC	CTCTGTCTGT	GTCTGGCACC	CTAAGTGGCT	TTGCATGGGC	100
ACAGGTTTCT	GCCCCCTGCAT	GGAGCTTAAA	TAGATCTTTC	TCCACAGGCC	150
TATACCCCTG	CATTGTAAGT	TTAAGTGGCT	TTATGTGGAT	ACAGGTCTCT	200
GCCCTTGTAT	GCAGGCCTAA	GTTTTCTGT	CTGCTTAACC	CCTCCAAGTG	250
AAGCTACTGA	AAGATCTAAC	CCACTTTTGG	AAGCTGAAA	CTAGACTTTT	300
ATGCGAGTGGC	CTAACAAAGTT	TTAATTTCTT	CCACAGGGTT	TGCAGAAAAG	350
AGCTTGATCC	ACGAGTTCAAG	AAGTCTGGT	ATGTTCTCTAG	AAAG	394
ATG TTC TCC	TGG AAA GCT	TCA AAA GCC	AGG TCT CCA	TTA AGT	436
CCA AGG TAT	TCT CTA CCT	GGT AGT ACA	GAG GTA CTT	ACA GGT	478
TGT CAT TCT	TAT CCT TCC	AGA TTC CTG	TCT GCC AGC	TCT TTT	520
ACT TCA GCC	CTG AGC ACA	GTC AAC ATG	CCT AGG GGT	CAA AAG	565
AGT AAG ACC	CGC TCC CGT	GCA AAA CGA	CAG CAG TCA	CGC AGG	604
GAG GTT CCA	GTA GTT CAG	CCC ACT GCA	GAG GAA GCA	GGG TCT	646
TCT CCT GTT	GAC CAG AGT	GCT GGG TCC	AGC TTC CCT	GGT GGT	688

TCT GCT CCT CAG GGT GTG AAA ACC CCT GGA TCT TTT GGT GCA	730
GGT GTA TCC TGC ACA GGC TCT GGT ATA GGT GGT AGA AAT GCT	772
GCT GTC CTG CCT GAT ACA AAA AGT TCA GAT GGC ACC CAG GCA	814
GGG ACT TCC ATT CAG CAC ACA CTG AAA GAT CCT ATC ATG AGG	856
AAG GCT AGT GTG CTG ATA GAA TTC CTG CTA GAT AAA TTT AAG	898
ATG AAA GAA GCA GTT ACA AGG AGT GAA ATG CTG GCA GTA GTT	940
AAC AAG AAG TAT AAG GAG CAA TTC CCT GAG ATC CTC AGG AGA	982
ACT TCT GCA CGC CTA GAA TTA GTC TTT GGT CTT GAG TTG AAG	1024
GAA ATT GAT CCC AGC ACT CAT TCC TAT TTG CTG GTA GGC AAA	1066
CTG GGT CTT TCC ACT GAG GGA AGT TTG AGT AGT AAC TGG GGG	1108
TTG CCT AGG ACA GGT CTC CTA ATG TCT GTC CTA GGT GTG ATC	1150
TTC ATG AAG GGT AAC CGT GCC ACT GAG CAA GAG GTC TGG CAA	1192
TTT CTG CAT GGA GTG GGG GTA TAT GCT GGG AAG AAG CAC TTG	1234
ATC TTT GGC GAG CCT GAG GAG TTT ATA AGA GAT GTA GTG CGG	1276
GAA AAT TAC CTG GAG TAC CGC CAG GTA CCT GGC AGT GAT CCC	1314
CCA AGC TAT GAG TTC CTG TGG GGA CCC AGA GCC CAT GCT GAA	1360
ACA ACC AAG ATG AAA GTC CTG GAA GTT TTA GCT AAA GTC AAT	1402
GGC ACA GTC CCT AGT GCC TTC CCT AAT CTC TAC CAG TTG GCT	1444
CTT AGA GAT CAG GCA GGA GGG GTG CCA AGA AGG AGA GTT CAA	1486
GGC AAG GGT GTT CAT TCC AAG GCC CCA TCC CAA AAG TCC TCT	1528
AAC ATG TAG	1537
TTGAGTCGTGT TCTGTTGTGT TTGAAAAACA GTCAGGCTCC TAATCAGTAG	1587
AGAGTTCAATA GCCTTACCGA ACCAACATGC ATCCATTCTT GGCCTGTTAT	1637
ACATTAGTAG AATGGAGGTG ATTTTTGTTA CTTTTCAAAT GTTTGTAA	1687
CTAAACAGTG CTTTTGCCA TGCTTCTTGT TAATCAGCATA AAGAGGTAAC	1737
TGTCACTTGT CAGATTAGGA CTTGTTTGT TATTGCAAC AAACCTGGAAA	1787
ACATTATTTT GTTTTACTA AAACATTGTG TAACATTGCA TTGGAGAAGG	1837
GATTGTCATG GCAATGTGAT ATCATACTAGT CGTGAAACAA CAGTGAAGTG	1887
GGAAAGTTA TATTGTTAAT TTTGAAAATT TTATGAGTGT GATTGCTGTA	1937
TACTTTTTTC TTTTTGTAT AATGCTAAGT GAAATAAAAGT TGGATTTGAT	1987
GACTTTACTC AAATTCATTA GAAAGTAAT CGTAAACACTC TATTACTTTA	2037
TTATTTCTT CAATTATGAA TTAAGCATTG GTTATCTGGA AGTTTCTCCA	2087
GTAGCACAGG ATCTAGTATG AAATGTATCT AGTATAGGCA CTGACAGTGA	2137
GTATCAGAG TCT	2150

## (2) INFORMATION FOR SEQUENCE ID NO: 25:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2099 base pairs
  - (B) TYPE: nucleic acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (ix) FEATURE:
  - (A) NAME/KEY: smage-II
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

ACCTTATTGG GTCTGTCTGC ATATGCCTCC ACTTGTGTGT AGCAGTCTCA	50
AATGGATCTC TCTCTACAGA CCTCTGCTCG TGTCTGGCAC CCTAAGTGGC	100
TTTGCATGGG CACAGGTTTC TGCCCCCTGCA TGGAGCTTAA ATAGATCTT	150
CTCCACAGGC CTATACCCCT GCATTGTAAG TTTAAGTGGC TTTATGTGGA	200
TACAGGTCTC TGCCCTTGTA TGCAAGGCTA AGTTTTCTG TCTGCTTAGC	250
CCCTCCAAGT GAAGCTAGTG AAAGATCTAA CCCACTTTG GAAGTCTGAA	300
ACTAGACTTT TATGCGTAGG CCTAACAAAGT TTTAATTCTC TCCACAGGGT	350
TTGCAGAAAAA GAGCTTGATC CACGAGTTCG GAAGTCTCG TATGTTCTA	400
GAAAGATGTT CTCCTGGAAA GCTTCAAAAG CCAGGTCTCC ATTAAGTCCA	450
AGGTATTCTC TACCTGGTAG TACAGAGGTA CTTACAGGTT GTCATTCCTA	500
TCTTTCCAGA TTCTGTCTG CCAGCTCTT TACTTCAGCC CTGAGCACAG	550
TCAACATGCC TAGGGGTCAA AAGAGTAAGA CCCGCTCCCG TGCAAAACGA	600
CAGCAGTCAC GCAGGGAGGT TCCAGTAGTT CAGCCCACTG CAGAGGAAGC	650
AGGGTCTCT CCTGTTGACC AGAGTGCTGG GTCCACCTTC CCTGGTGGTT	700
CTGCTCTCA GGGTGTGAAA ACCCTGGAT CTTTGGTGCA AGGTGTATCC	750
TGCACAGGCT CTGGTATAGG TGGTAGAAAT GCTGCTGTCC TGCCTGATAC	800
AAAAAGTTCA GATGGCACCC AGGCAGGGAC TTCCATTCTAG CACACACTGA	850
AAGATCCTAT CATGAGGAAG GCTAGTGTGC TGATAGAATT CCTGCTAGAT	900

AAAGTTTAAGA	TGAAAAGAAGC	AGTTACAAGG	AGTGAATGCG	TGGCAGTAGT	950
TAACAAGAAG	TATAAGGAGC	AATTCCCTGA	GATCCTCA	G AGAACTTCTG	1000
CACGCCTAGA	ATTAGCTTT	GGTCTTGAGT	TGAAGGAAT	TGATCCCAGC	1050
ACTCATTCT	ATTGCTGGT	AGGCAAACGT	GGTCTTCCA	CTGAGGGAAAG	1100
TTTGAGTAGT	AACTGGGGGT	TGCCTAGGAC	AGGTCTCCTA	ATGTCTGTCC	1150
TAGGTGTGAT	CTTCATGAAG	GGTAACCGTG	CCACTGAGCA	AGAGGTCTGG	1200
CAATTCTGC	ATGGAGTGGG	GGTATATGCT	GGGAAGAAGC	ACTTGATCTT	1250
TGGCGAGCCT	GAGGAGTTA	TAAGAGATGT	AGTGCGGGAA	AATTACCTGG	1300
AGTACCGCCA	GGTACCTGGC	AGTGAATCCC	CAAGCTATGA	TTTCCGTG	1350
GGACCCAGAG	CCCATGCTGA	AAACAACCAAG	ATGAAAGTCC	TGGAAGTTTT	1400
AGCTAAAGTC	AATGGCACAG	TCCCTAGTGC	CTTCCCTAAT	CTCTACCAGT	1450
TGGCTCTTAG	AGATCAGGCA	GGAGGGGTGC	CAAGAAGGAG	AGTTCAAGGC	1500
AAGGGTGTTC	ATTCCAAGGC	CCCATCCCAA	AACTCTCTA	ACATGTTAGTT	1550
GAGTCTGTTC	TGTTGTGTT	AAAAAACAGT	CAGGCTCCTA	ATCAGTAGAG	1600
AGTTCATAGC	CTACCAAGAAC	CAACATGCA	CCATTCTGG	CCTGTTATAC	1650
ATTAGTAGAA	TGGAGGCTAT	TTTGTACT	TTTCAATGT	TTGTTTAAC	1700
AAACAGTGC	TTTGCCATG	CTTCTGTTA	ACTGCATAAA	GAGGTAAC	1750
TCACCTGTCA	GATTAGGACT	TGTTTGTAA	TTTCAACAA	ACTGGAAAAC	1800
ATTATTTTGT	TTTACTAAA	ACATTGTGTA	ACATTGCA	GGAGAAGGG	1850
TTGTCATGGC	AATGTGATAT	CATACAGTGG	TGAAACAACA	GTGAAGTGGG	1900
AAAGTTTATA	TTGTTAGTTT	TGAAAATTTT	ATGAGTGTGA	TTGCTGTATA	1950
CTTTTTTCTT	TTTGTATAA	TGCTAAGTGA	AATAAAGTTG	GATTGATGA	2000
CTTTACTCAA	ATTCATTAGA	AAAGTAAATCA	AAAACCTCA	TTACTTTATT	2050
ATTTTCTTCA	ATTATTAATT	AAGCATTGGT	TATCTGGAAG	TTTCTCCAG	2099

## (2) INFORMATION FOR SEQUENCE ID NO: 26:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acids
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Glu Ala Asp Pro Thr Gly His Ser Tyr

5

## (2) INFORMATION FOR SEQUENCE ID NO: 27:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

TGGAGGACCA GAGGCC

19

## (2) INFORMATION FOR SEQUENCE ID NO: 28:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GGACGATTAT CAGGAGGCCT GC

22

87

## (2) INFORMATION FOR SEQUENCE ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GAGCAGACAG GCCAACCG

18

## (2) INFORMATION FOR SEQUENCE ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

AAGGACTCTG CGTCAGGC

18

## (2) INFORMATION FOR SEQUENCE ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

CTAGAGGAGC ACCAAAGGAG AAG

23

## (2) INFORMATION FOR SEQUENCE ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

TGCTCGAAC ACAGACTCTG G

21

## (2) INFORMATION FOR SEQUENCE ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

TGGAGGACCA GAGGCC

19

88

## (2) INFORMATION FOR SEQUENCE ID NO: 34:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CAGGATGATT ATCAGGAAGC CTGT

24

## (2) INFORMATION FOR SEQUENCE ID NO: 35:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CAGAGGAGCA CCGAAGGAGA A

21

## (2) INFORMATION FOR SEQUENCE ID NO: 36:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

CAGGTGACCG GGGTGTGTC

19

## (2) INFORMATION FOR SEQUENCE ID NO: 37:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

CCCCAGAGAA GCACTGAAGA AG

22

## (2) INFORMATION FOR SEQUENCE ID NO: 38:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

GGTGAGCTGG GTCCGGG

17

89

## (2) INFORMATION FOR SEQUENCE ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

CCCCAGAGCA GCACTGACG

19

## (2) INFORMATION FOR SEQUENCE ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

CAGCTGAGCT GGGTCGACC

19

## (2) INFORMATION FOR SEQUENCE ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

CACAGAGCAG CACTGAAGGA G

21

## (2) INFORMATION FOR SEQUENCE ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

CTGGGTAAAG ACTCACTGTC TGG

23

## (2) INFORMATION FOR SEQUENCE ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GAGAACCCAG AGGATCACTG GA

22

90

## (2) INFORMATION FOR SEQUENCE ID NO: 44:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

GGGAAAAGGA CTCAGGTCT ATC

23

## (2) INFORMATION FOR SEQUENCE ID NO: 45:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

GGTGGAAAGTG GTCCGCATCG

20

## (2) INFORMATION FOR SEQUENCE ID NO: 46:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

GCCCTCCACT GATCTTTAGC AA

22

## (2) INFORMATION FOR SEQ ID NO: 47:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

CGGCCGAAGG AACCTGACCC AG

22

## (2) INFORMATION FOR SEQ ID NO: 48:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

GCTGGAACCC TCACTGGTTT GCC

23

91

(2) INFORMATION FOR SEQ ID NO: 49:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

AAGTAGGACC CGAGGCAGTG

20

(2) INFORMATION FOR SEQ ID NO: 50:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

GAAGAGGAAG AAGCGGTCTG

20

(2) INFORMATION FOR SEQ ID NO: 51:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

TGGAGGGACCA GAGGCC

19

(2) INFORMATION FOR SEQ ID NO: 52:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

GGACGATTAT CAGGAGGCCT GC

22

(2) INFORMATION FOR SEQ ID NO: 53:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

ACTCAGCTCC TCCCAGATTT

20

92

(2) INFORMATION FOR SEQ ID NO: 54:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

GAAGAGGAGG GGCCAAG

17

(2) INFORMATION FOR SEQ ID NO: 55:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

TCTTGTATCC TGGAGTCC

18

(2) INFORMATION FOR SEQ ID NO: 56:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

TTGCCAAGAT CTCAGGAA

18

Claims:

1. Isolated nucleic acid molecule useful as a primer in specifically determining expression of a member of the MAGE family of tumor rejection antigen precursor.
2. The isolated nucleic acid molecule of claim 1, selected from the group consisting of one of SEQ ID NOS: 27-48.
3. A kit useful in determining expression of a MAGE tumor rejection antigen precursor, comprising at least one part of SEQ ID NOS: 27 and 28, 29 and 30, 31 and 32, 33 and 34, 35 and 36, 37 and 38, 39 and 40, 41 and 42, 43 and 44, 45 and 46, and 47-48.
4. Method for determining expression of a MAGE tumor rejection antigen precursor in a cell comprising contacting said cell sample with at least one of the nucleic acid molecules of claim 2 and determining hybridization of said nucleic acid molecule to a target as a determination of expression of MAGE tumor rejection antigen precursor.
5. Method of claim 3, wherein said expression of said tumor rejection antigen precursor is a determination of presence, regression of spread of cancer.
6. The method of claim 5, wherein said cancer is melanoma.
7. The method of claim 5, wherein said cancer is lung adenocarcinoma, said method comprising contacting said sample with a pair of: SEQ ID NOS: 27 and 28, SEQ ID NOS: 47 and 48, or SEQ ID NOS: 49 and 50.

8. The method of claim 4, wherein said cancer is a head squamous cell carcinoma, a neck squamous cell carcinoma, a prostate carcinoma, and a bladder tumor, the method comprising contacting sample with SEQ ID NOS: 27 AND 28, SEQ ID NOS: 29 and 30, SEQ ID NOS: 47 and 48, or SEQ ID NOS: 49 and 50.
9. The method of claim 4, wherein said cancer is a bladder tumor, the method comprising contacting said sample with SEQ ID NOS: 47 and 48, SEQ ID NOS: 49 and 50, or SEQ ID NOS: 51 and 52, followed by amplification.
10. The method of claim 4, wherein said MAGE tumor rejection antigen precursor is MAGE-1, MAGE-2, MAGE-3 or MAGE-4.

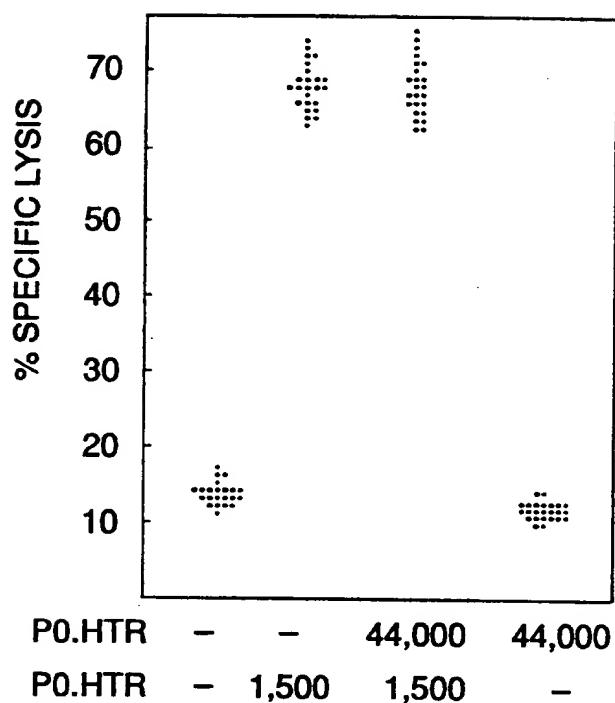
## AMENDED CLAIMS

[received by the International Bureau on 27 June 1995 (27.06.95);  
original claims 1-10 replaced by amended claims 1,9 (2 pages)]

1. Isolated nucleic acid molecule useful as a primer in specifically determining expression of a member of the MAGE group of tumor rejection antigen precursors, selected from the group consisting of SEQ ID NOS: 27-46.
2. Kit useful in determining expression of a MAGE tumor rejection antigen precursor, comprising at least one pair of:  
SEQ ID NOS: 27 and 28  
SEQ ID NOS: 29 and 30  
SEQ ID NOS: 31 and 32  
SEQ ID NOS: 33 and 34  
SEQ ID NOS: 35 and 36  
SEQ ID NOS: 37 and 38  
SEQ ID NOS: 39 and 40  
SEQ ID NOS: 41 and 42  
SEQ ID NOS: 43 and 44  
SEQ ID NOS: 45 and 46.
3. Method for determining expression of a MAGE tumor rejection antigen precursor in a cell comprising contacting said cell sample with at least one isolated nucleic acid molecule of claim 1 and determining hybridization of said nucleic acid molecule to a target as a determination of expression of MAGE tumor rejection antigen precursor.
4. The method of claim 3, wherein said expression of said tumor rejection antigen precursor is a determination of presence, regression or spread of cancer.
5. The method of claim 4, wherein said cancer is melanoma.

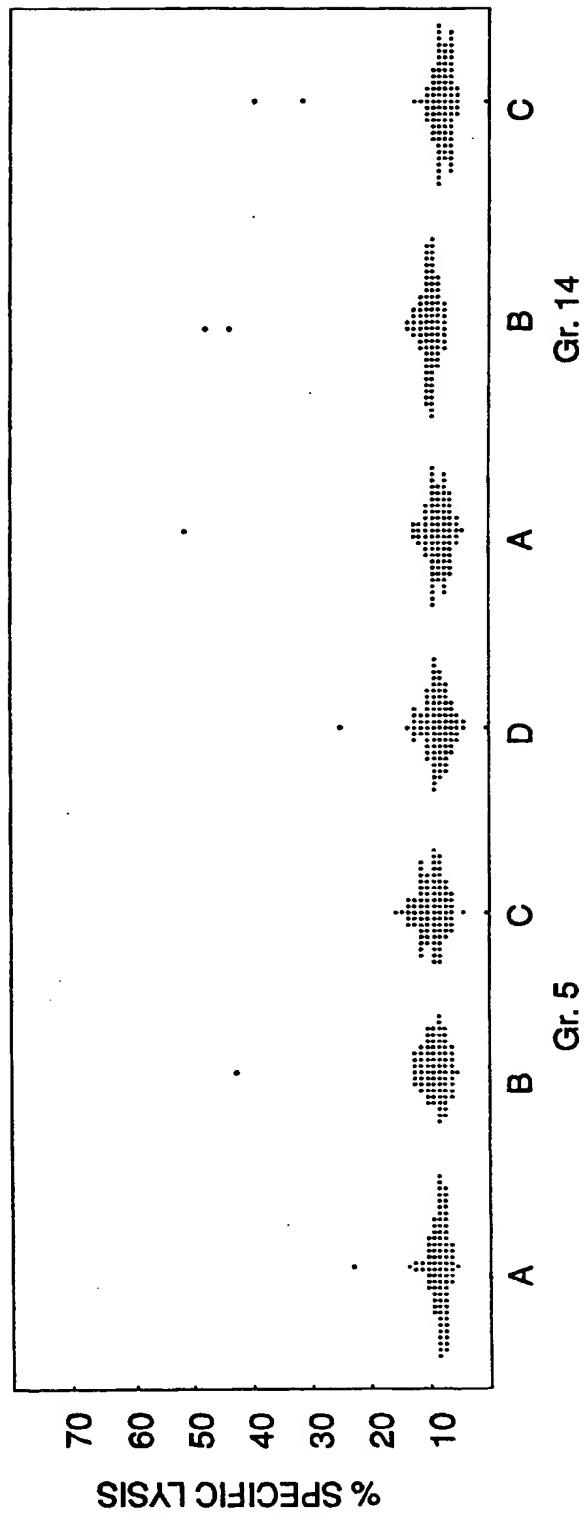
6. The method of claim 4, comprising contacting said sample with SEQ ID NOS: 27 and 28.
7. The method of claim 4, wherein said cancer is a head squamous cell carcinoma, a neck squamous cell carcinoma, a prostate carcinoma and a bladder tumor, the method comprising contacting said sample with SEQ ID NOS: 27 and 28 or SEQ ID NOS: 29 and 30.
8. The method of claim 4, wherein said cancer is a bladder tumor, said method comprising contacting said sample with SEQ ID NOS: 51 and 52, followed by amplification.
9. The method of claim 4, wherein said MAGE tumor rejection antigen precursor is MAGE-1, MAGE-2, MAGE-3 or MAGE-4.

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**FIG. 1A**

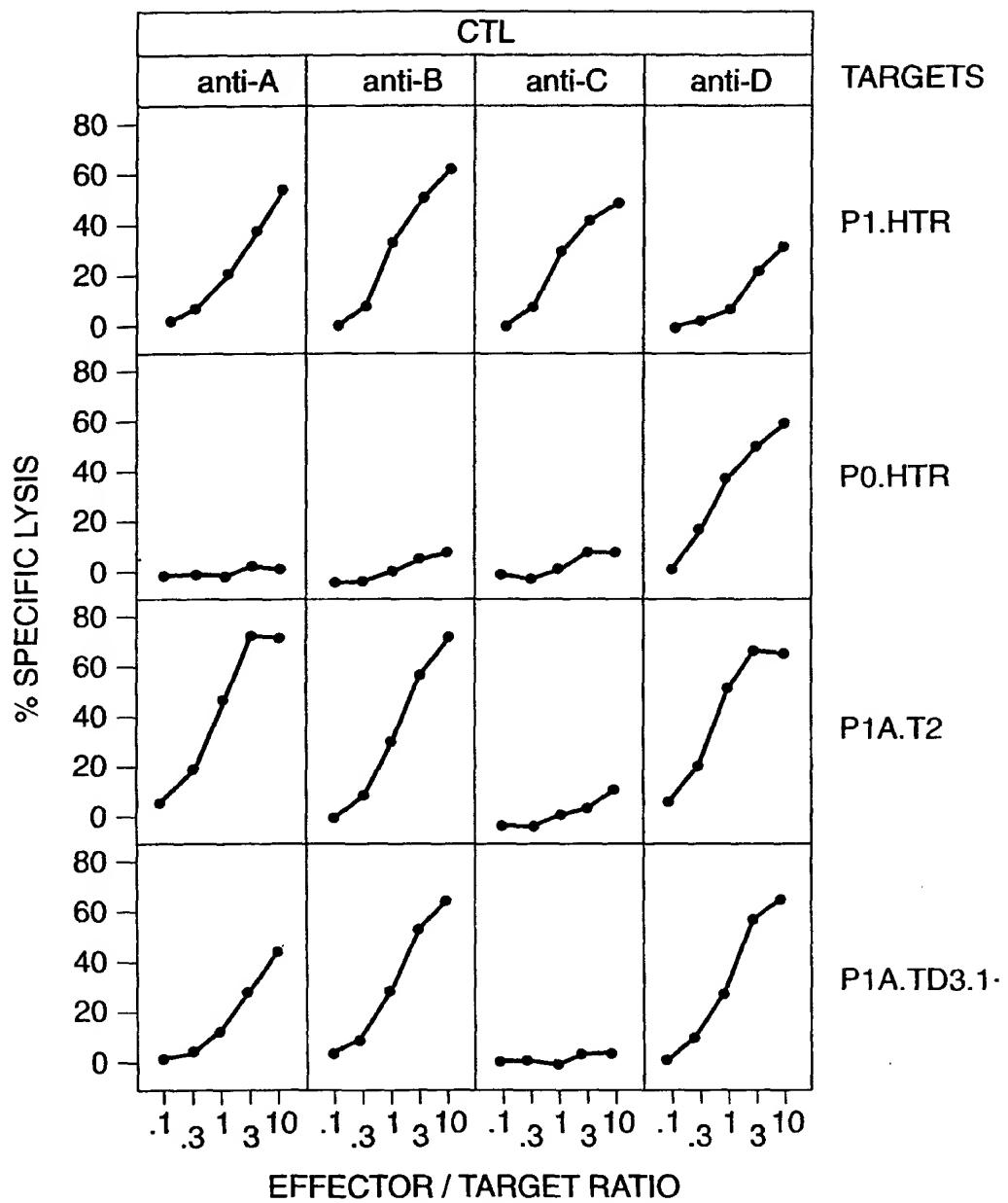
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FIG. 1B

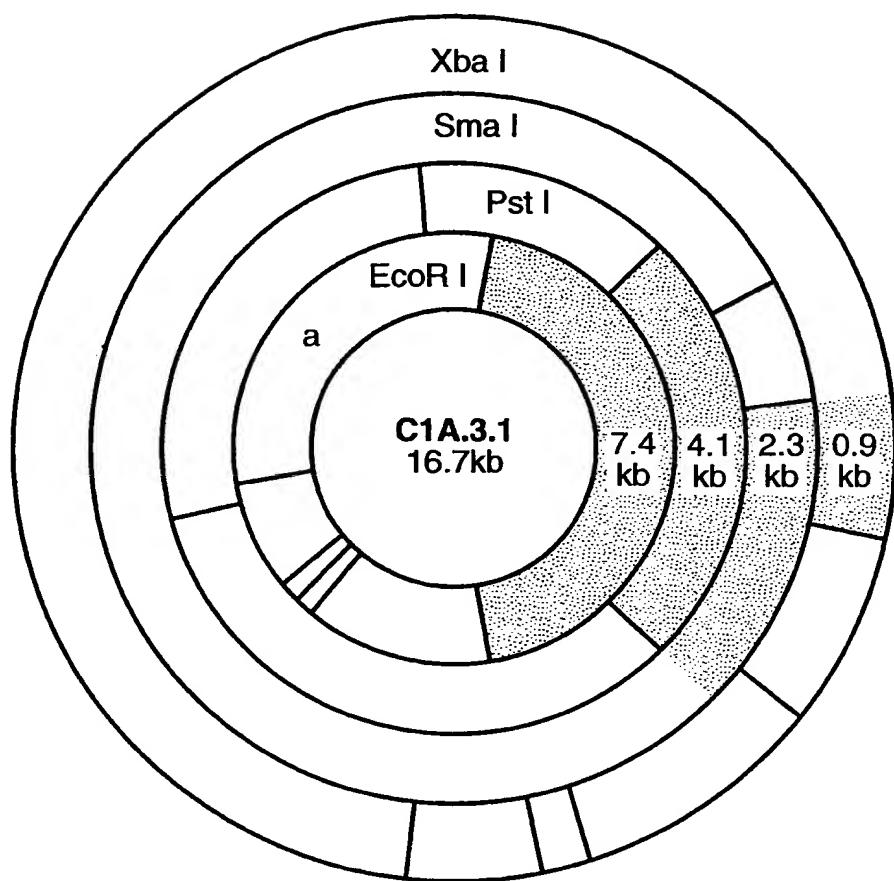


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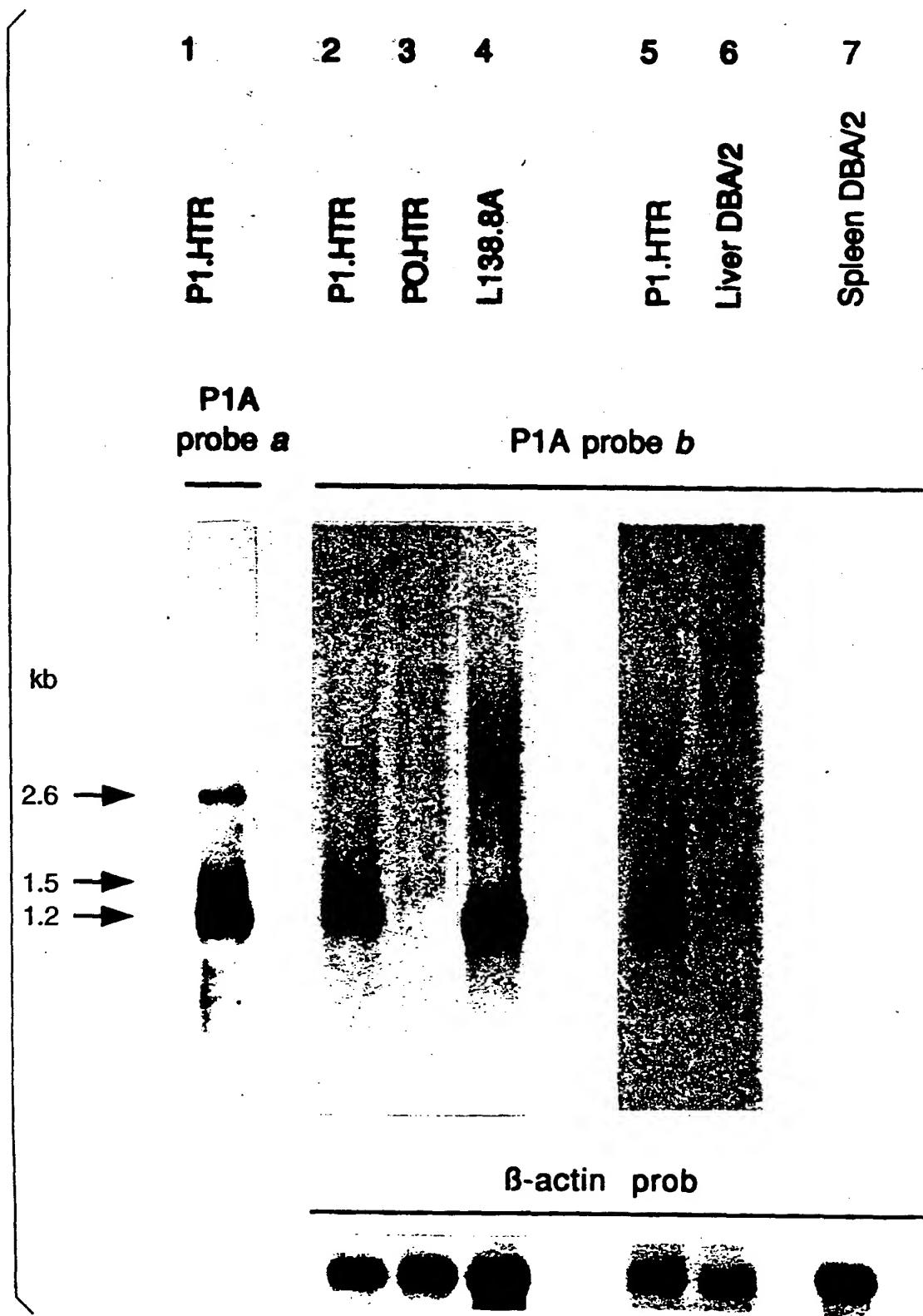
**FIG. 2**

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**FIG. 3**

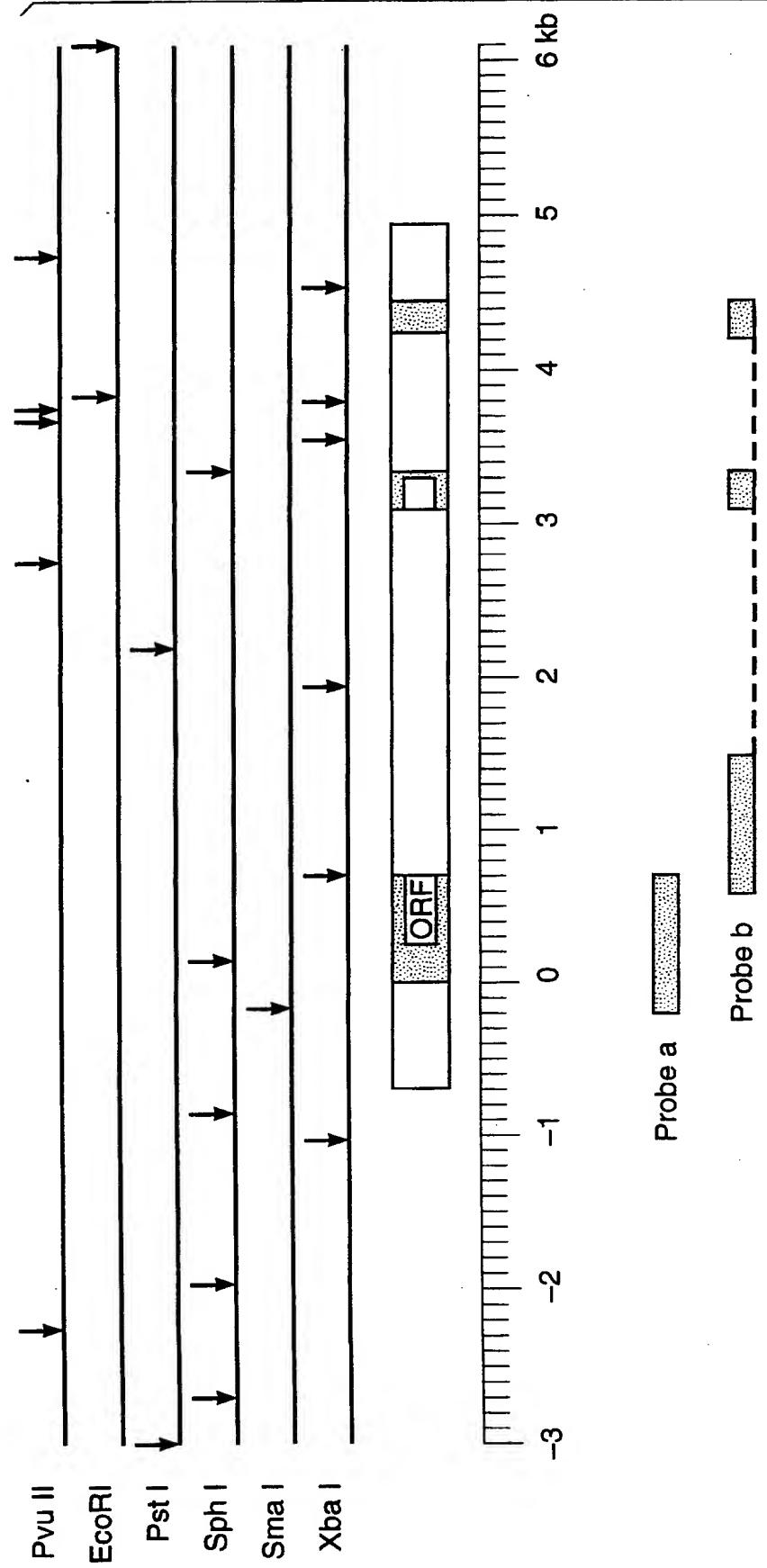
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## FIG. 4

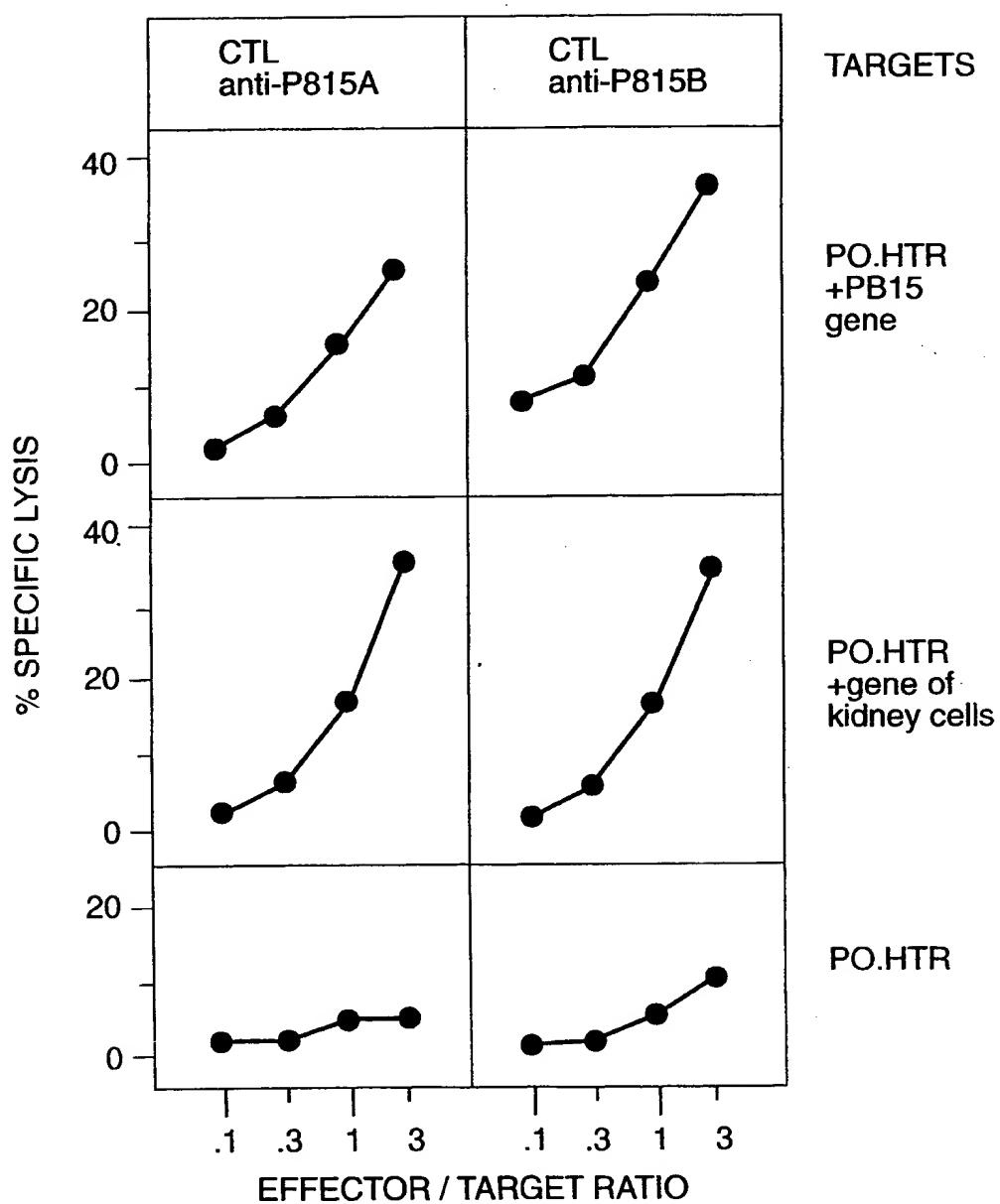


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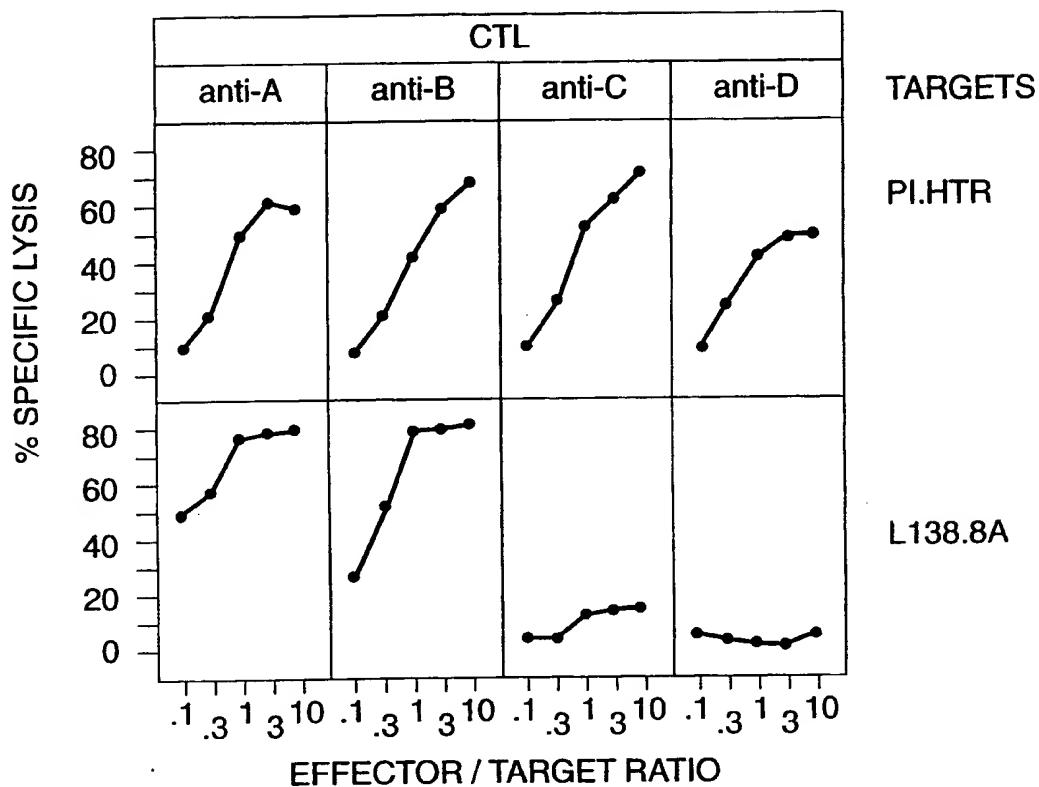
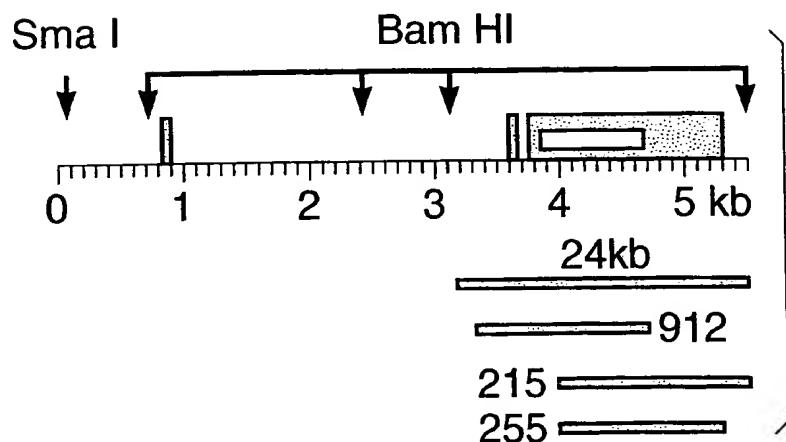
FIG. 5



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**FIG. 6**

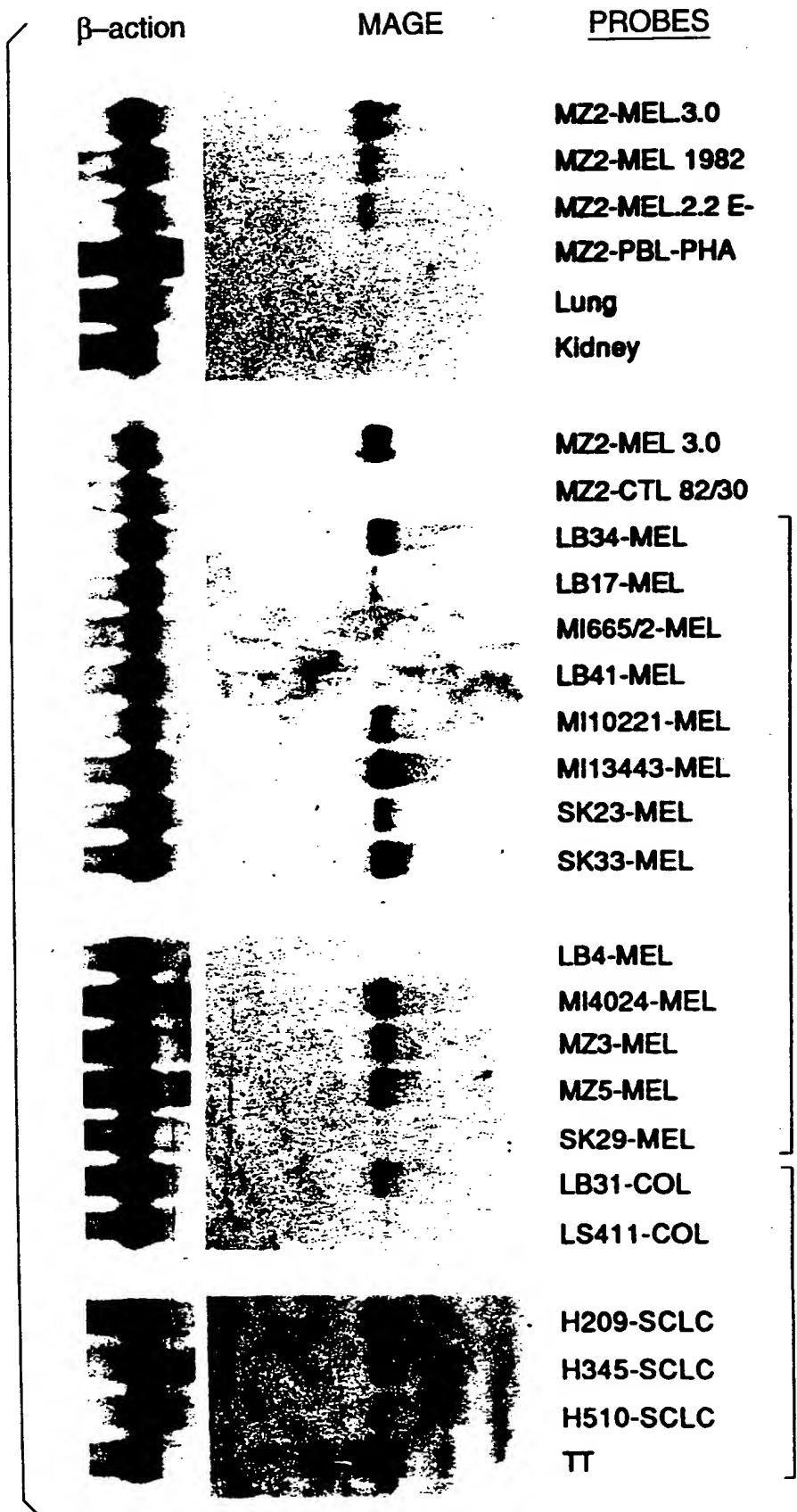
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**FIG. 7****FIG. 8**

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**FIG. 10**

Other melanomas

Other tumors

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**FIG. 11A**

		EXPRESSION OF MAGE GENE FAMILY				RECOGNITION BY ANI-E CTL		
		cDNA-PCR product probed with oligonucleotide specific for:				tested by:		
		MAGE-1	MAGE-2	MAGE-3†		TNF	release‡	Lysis§
Cells of patient MZ2	Northern blot probed with cross-reactive MAGE-1 probe*	+++	+++	+++		+	+	+
	melanoma cell line MZ2-MEL 3.0	+	+++	+++		+	+	+
	tumor sample MZ2 (1982)	+	+++	+++		+	+	+
	antigen-loss variant MZ2-MEL 2.2	+	-	+++		-	-	-
	CTL clone MZ2-CTL 82/30	-	-	-		-	-	-
	PHA-activated blood lymphocytes	-	-	-		-	-	-
Normal tissues	Liver	-	-	-		-	-	-
	Muscle	-	-	-		-	-	-
	Skin	-	-	-		-	-	-
	Lung	-	-	-		-	-	-
	Brain	-	-	-		-	-	-
	Kidney	-	-	-		-	-	-
Melanoma cell lines of HLA-A1 patients	LB34-MEL	++	+++	+++		+	+	-
	MI665/2-MEL	-	-	-		-	-	-
	MI10221-MEL	-	++	+++		-	-	-
	MI13443-MEL	++	+++	+++		+	+	+
	SK33-MEL	++	+++	+++		-	-	-
	SK23-MEL	++	+++	+++		-	-	-

\* Data obtained in the conditions of figure 5.

† Data obtained as described in figure 6.

‡ TNF release by CTL 82/30 after stimulation with the tumor cells as described in (11).

§ Lysis of 51 Cr labelled target by CTL 82/30 in the conditions of figure 1.

\*\* Cells transfected with the 2.4 kb fragment of gene MAGE-1 were tested for their ability to stimulate TNF release by CTL 82/30

**FIG. 11B**

EXPRESSION OF MAGE GENE FAMILY		RECOGNITION BY ANI-E CTL			tested by: TNF release† MAGE-3‡ lysis§	Expression of antigen MZ2-E after transfection**
		Northern blot probed with cross-reactive MAGE-1 probe*	cDNA-PCR product probed with oligonucleotide specific for: MAGE-1	MAGE-2		
Melanoma cell lines of other patients		LB17-MEL LB33-MEL LB4-MEL LB41-MEL M14024-MEL SK29-MEL MZ3-MEL MZ5-MEL	+	+	+++ +++ - - - - - -	- - - - - - - -
Melanoma tumor sample	BB5-MEL		+	+	+++ ++ - -	- - - -
Other tumor cell lines		small cell lung cancer H209 small cell lung cancer H345 small cell lung cancer H510 small cell lung cancer LB11 bronchial squamous cell carcinoma LB37 thyroid medullary carcinoma TT colon carcinoma LB31 colon carcinoma LS411	+++ +++ + + + - -	+++ +++ +++ +++ +++ - - -	- - - - - - - -	
Other tumor samples	chronic myeloid leukemia LLC5 acute myeloid leukemia TA		- -	- -	- -	- -

\* Data obtained in the conditions of figure 5.

† Data obtained as described in figure 6.

‡ TNF release by CTL 82/30 after stimulation with the tumor cells as described in (11).

§ Lysis of 51 Cr labelled target by CTL 82/30 in the conditions of figure 1.

\*\* Cells transfected with the 2.4 kb fragment of gene MAGE-1 were tested for their ability to stimulate TNF release by CTL 82/30

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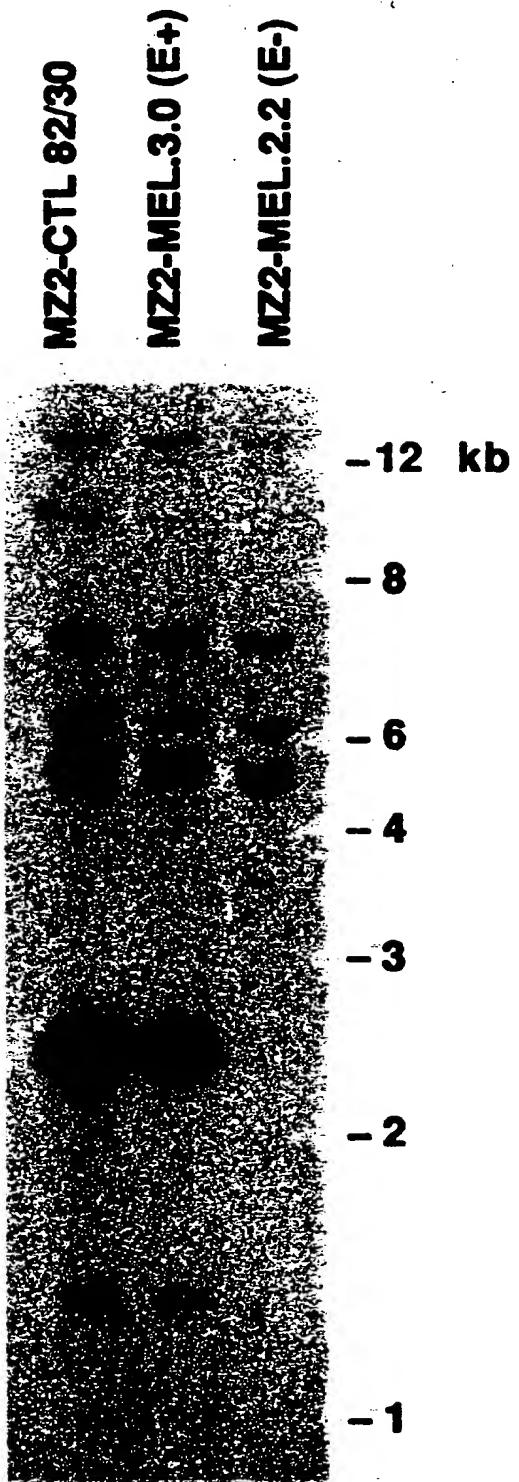
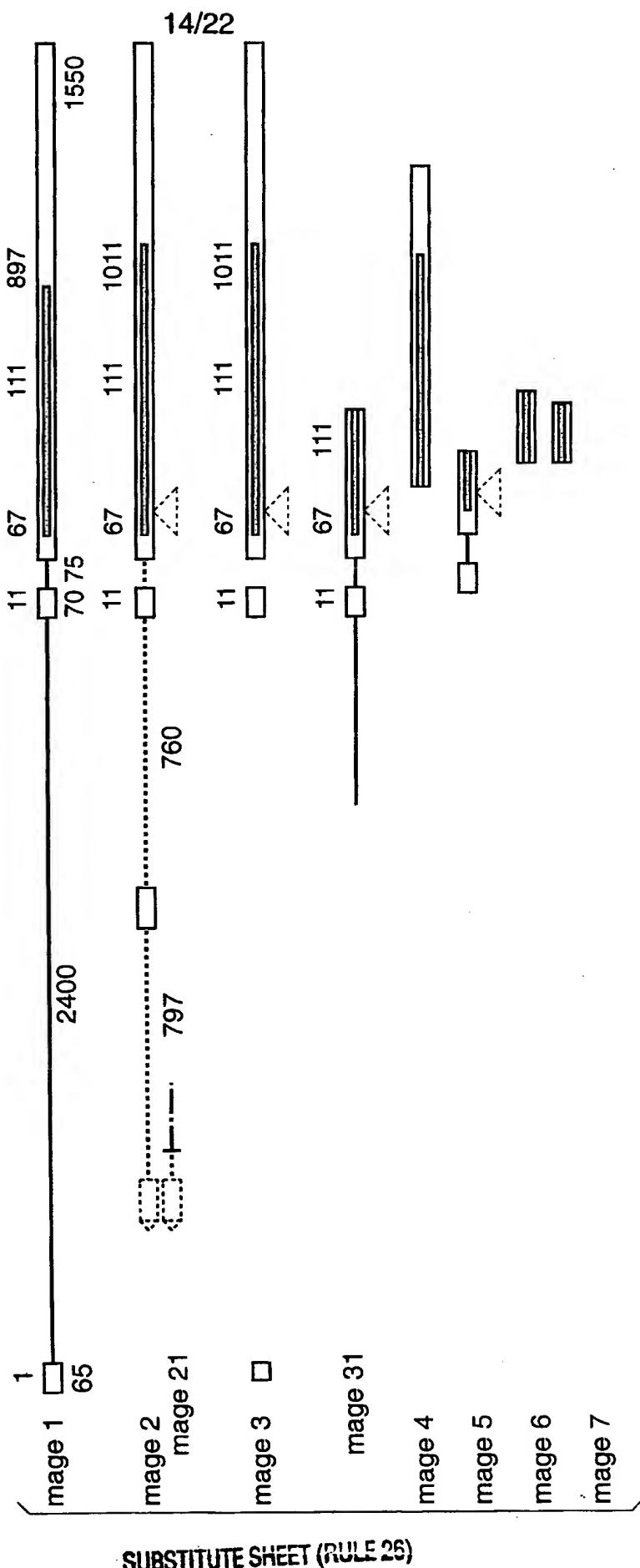
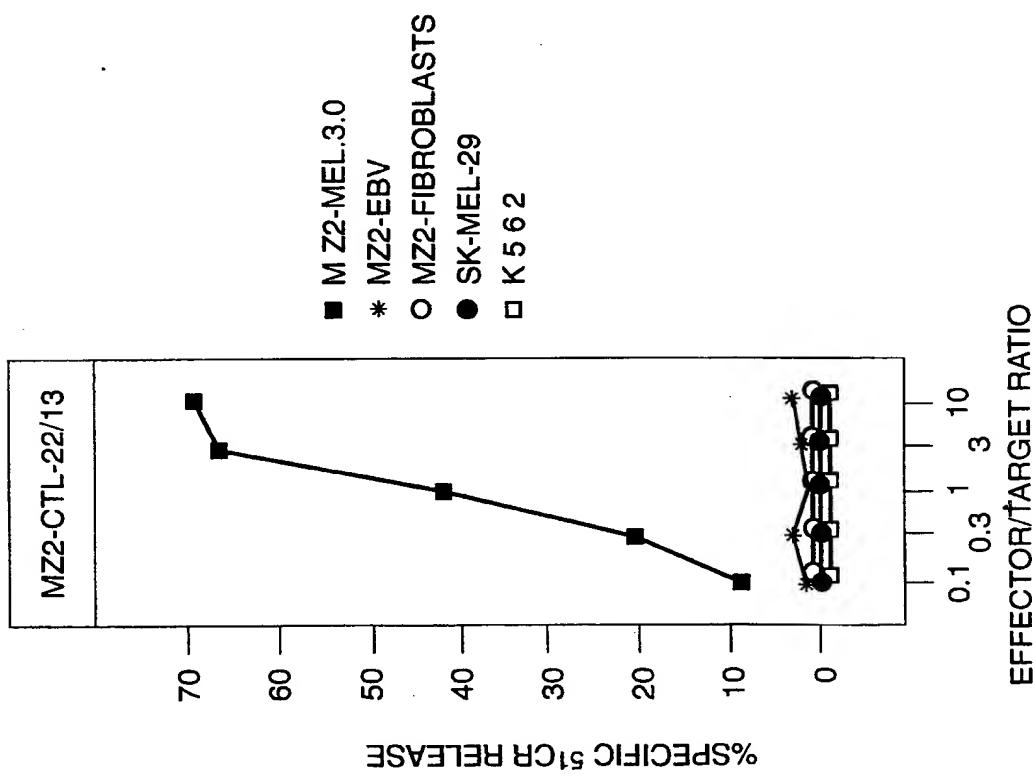
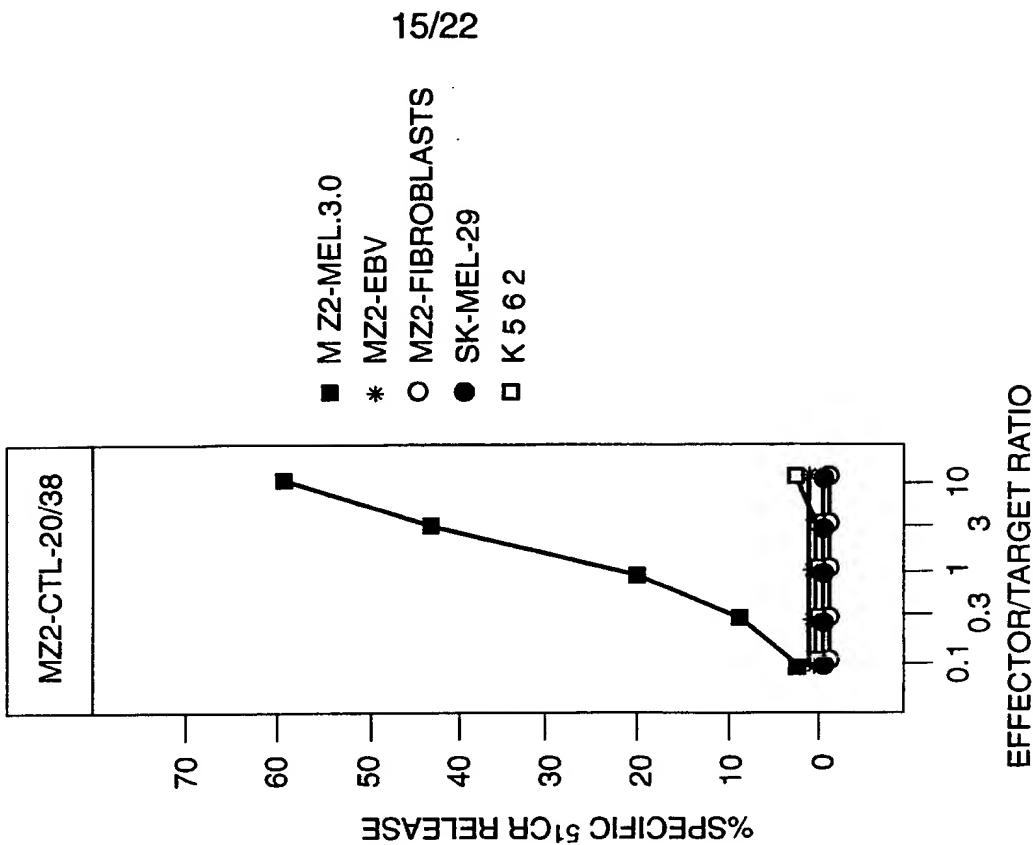
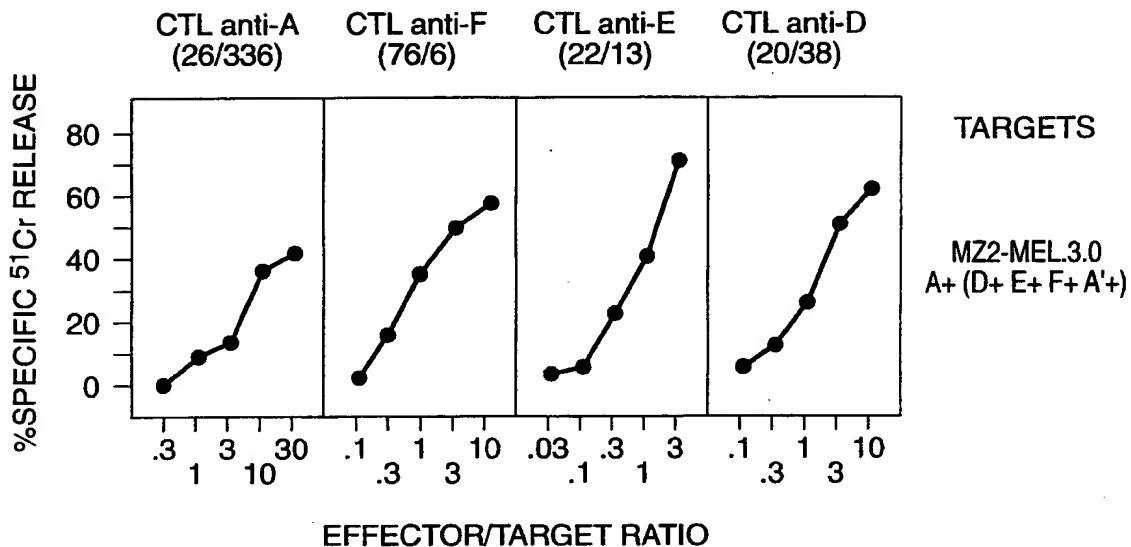
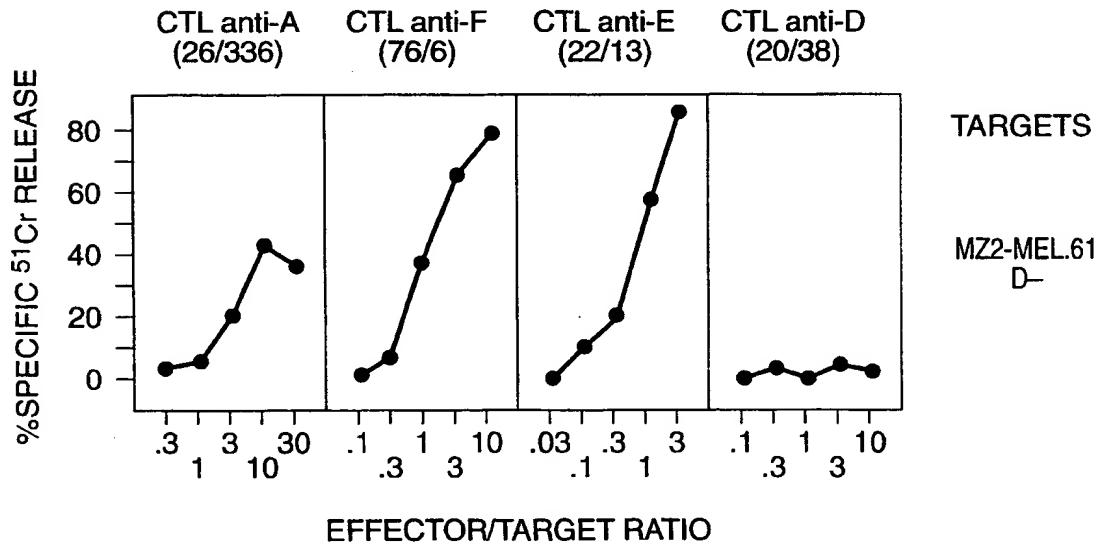
**FIG. 12**

FIG. 13

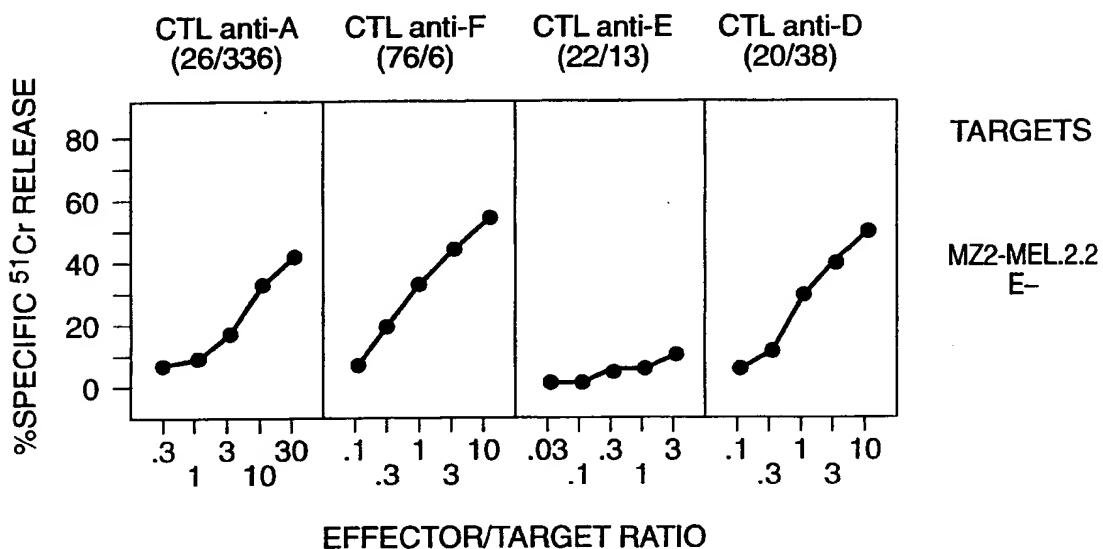
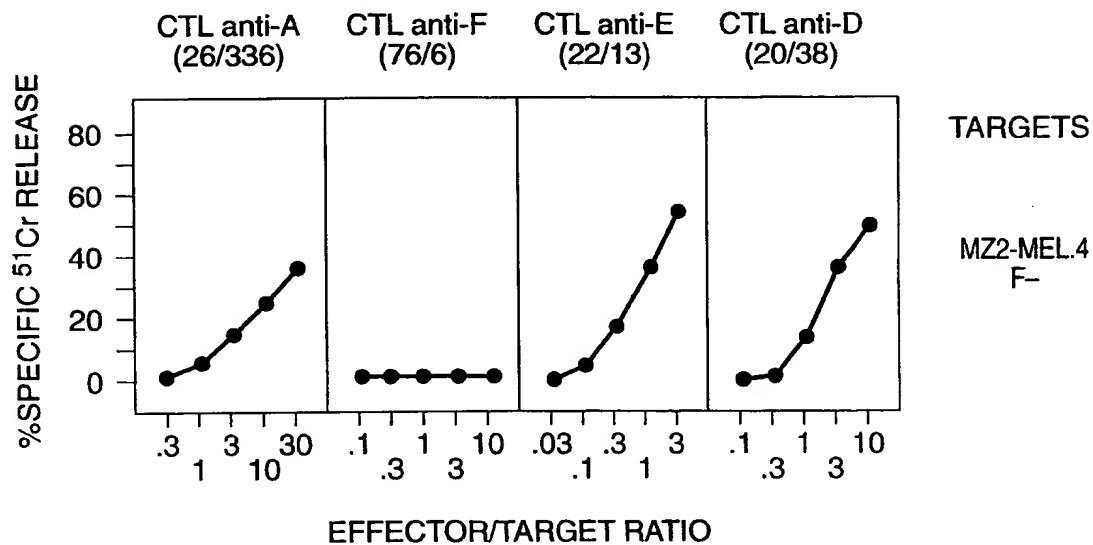


**FIG. 14A****FIG. 14B**

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**FIG. 15A****FIG. 15B**

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**FIG. 15C****FIG. 15D**

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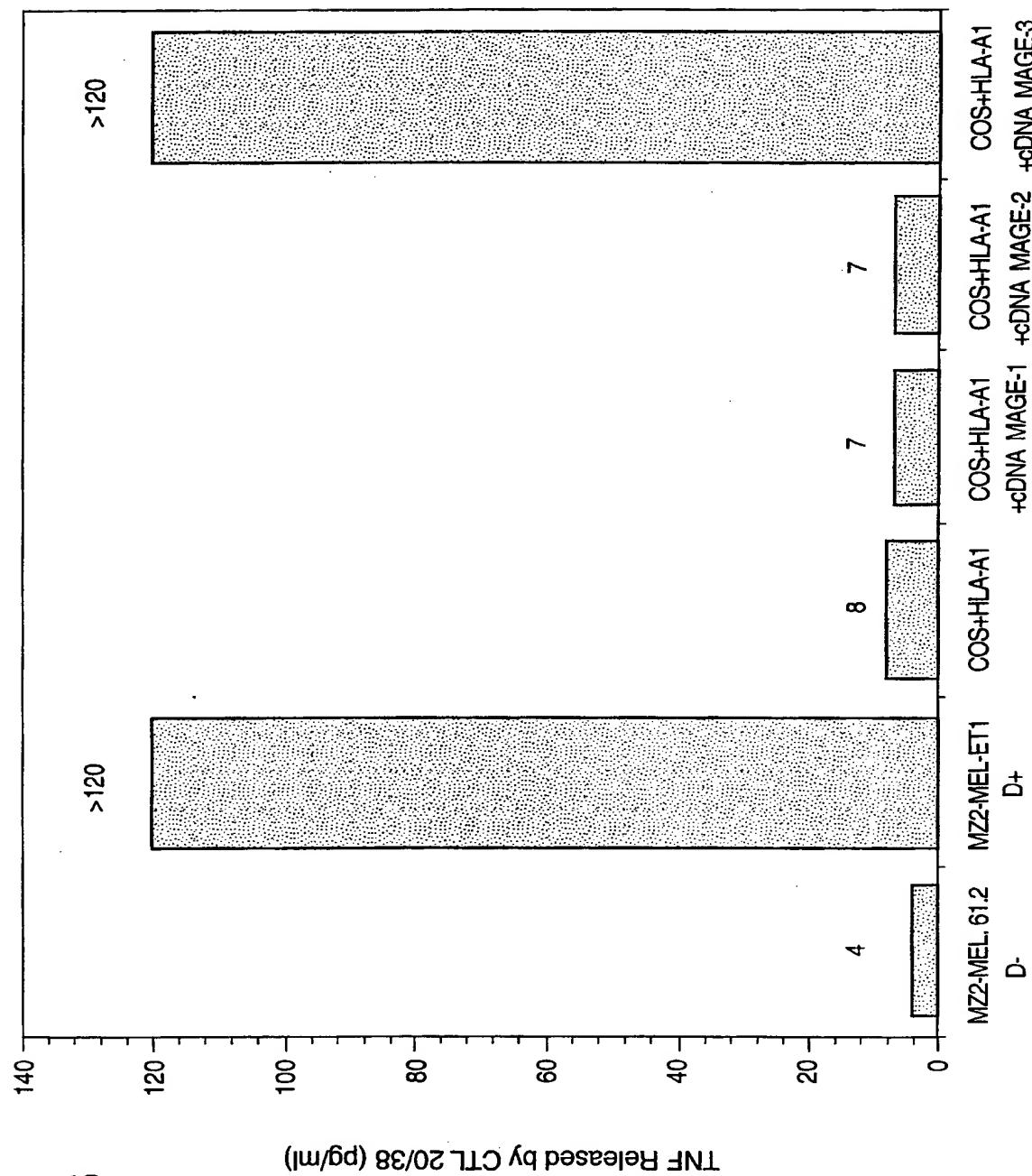
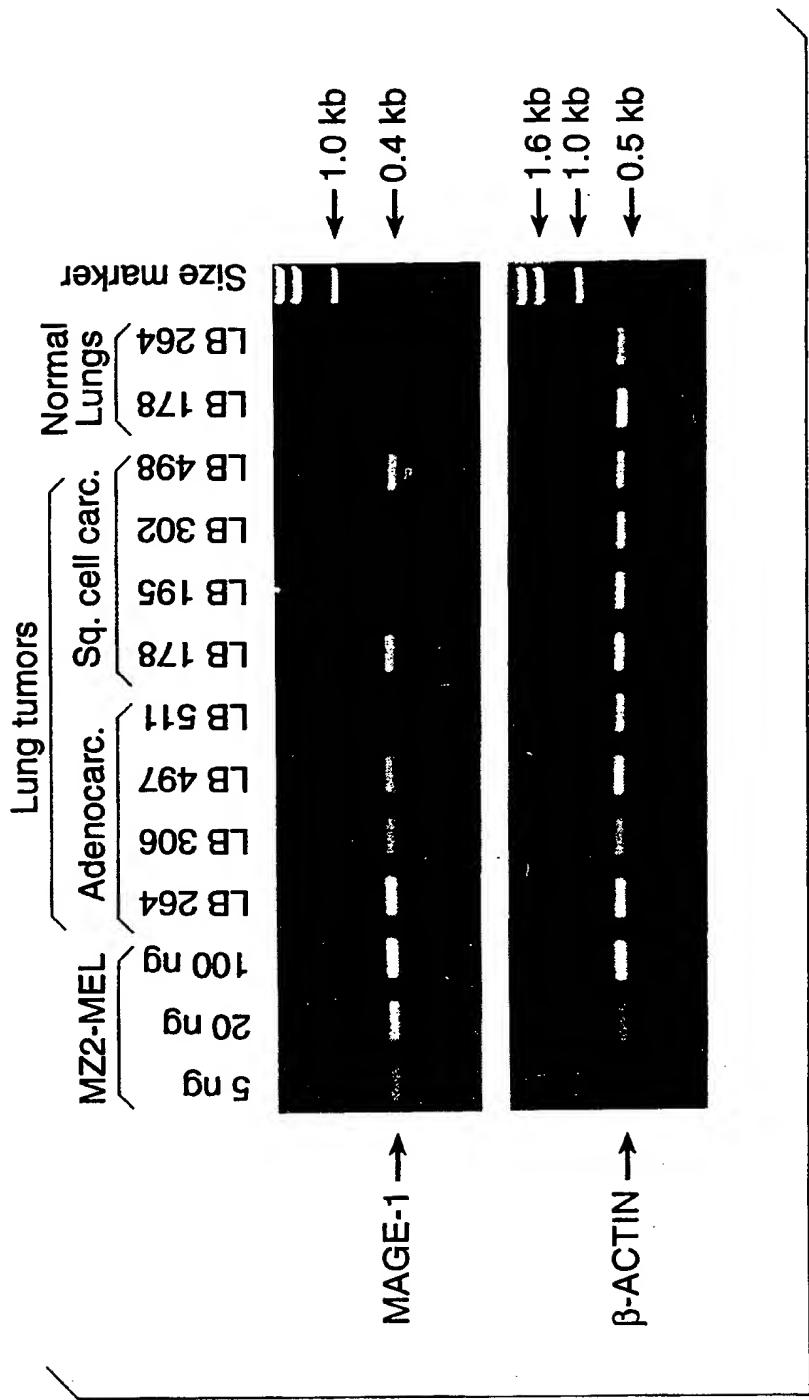
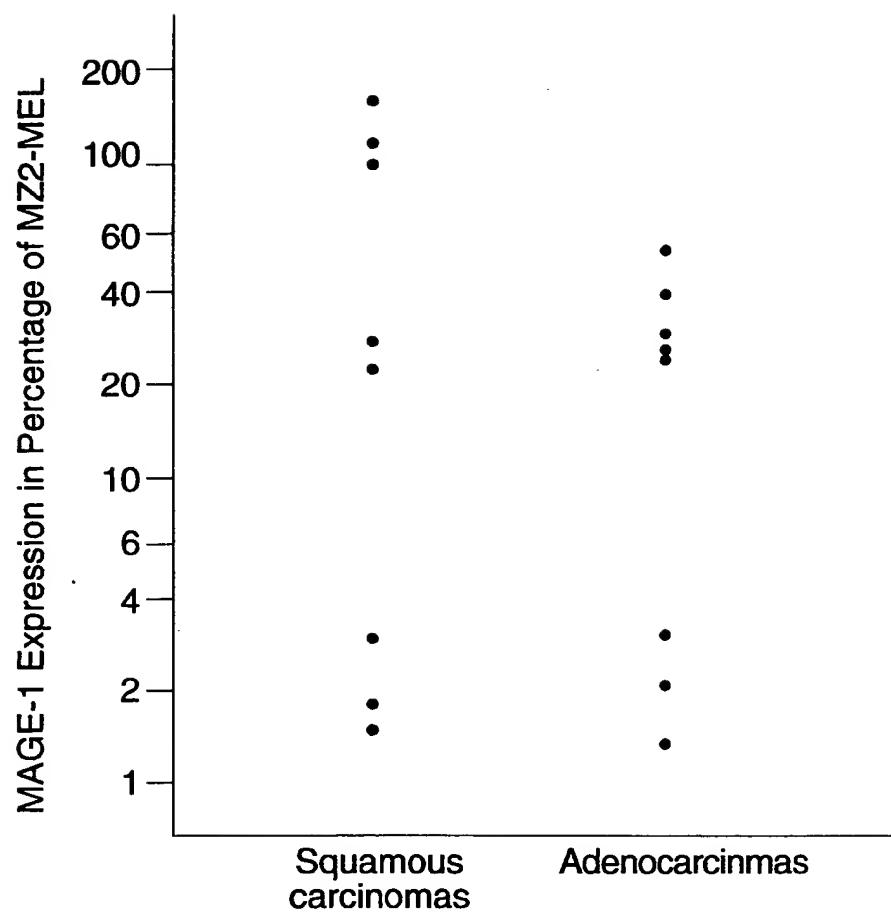


FIG. 16

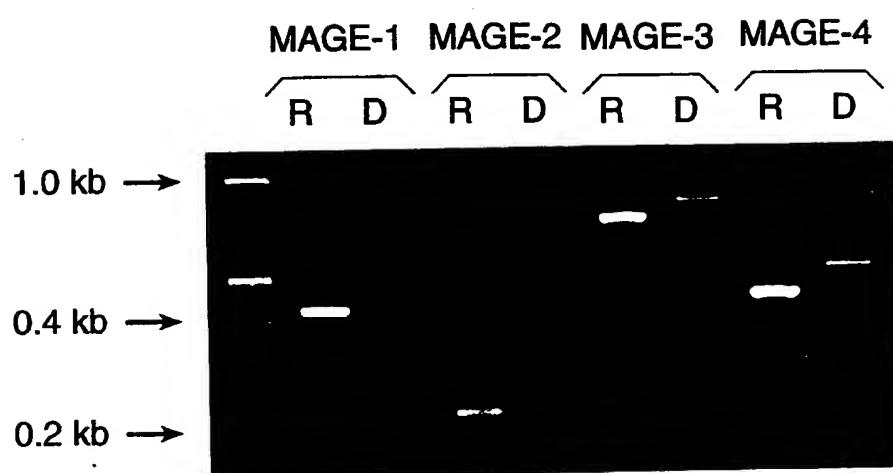
**FIG. 17**



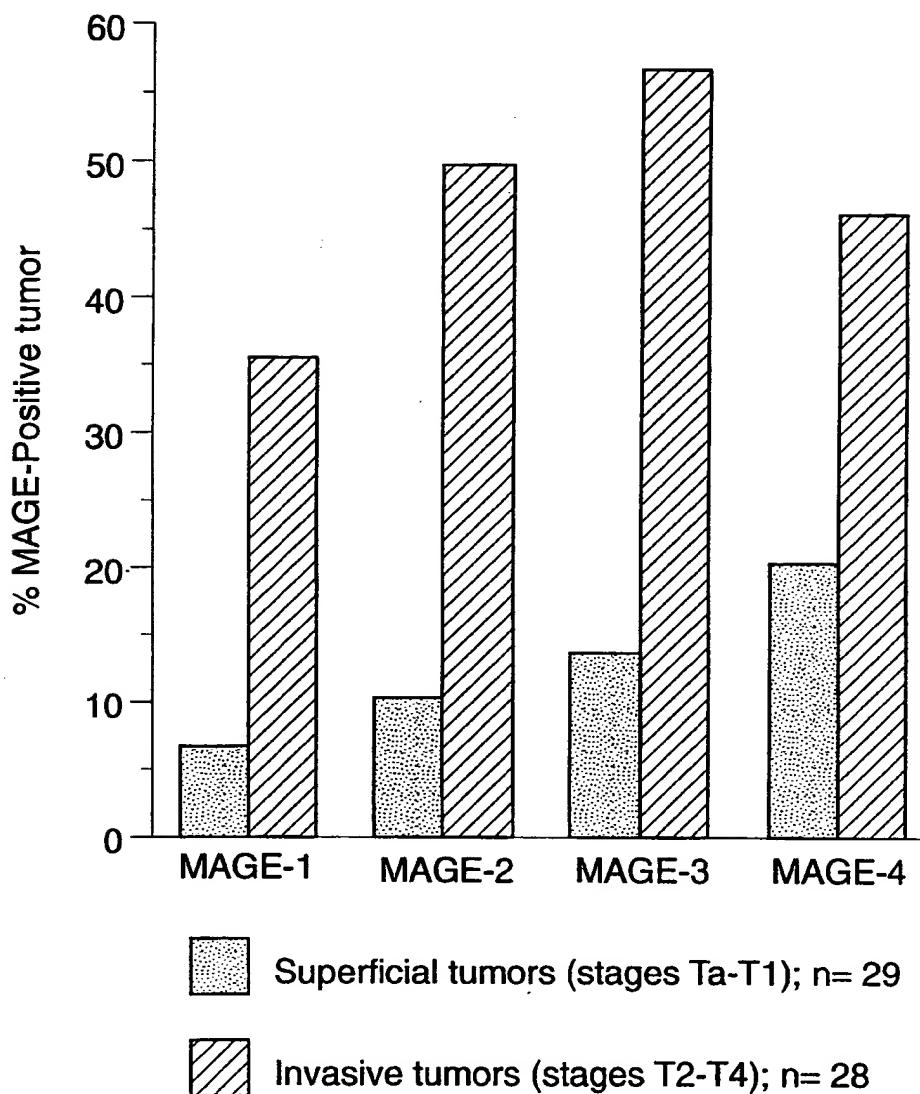
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**FIG. 18**

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**FIG. 19**

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**FIG. 20**

SUBSTITUTE SHEET (RULE 26)

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/02203

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68

US CL :435/6

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, BIOSIS, APS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	IMMUNOGENETICS, VOLUME 39, ISSUED 1994, SMET ET AL. "SEQUENCE AND EXPRESSION PATTERN OF HUMAN MAGE2 GENE", PAGES 121-129, SEE ENTIRE DOCUMENT.	1-10
Y	INTERNATIONAL JOURNAL OF CANCER, ISSUED 1994, WEYNANTS ET AL, "EXPRESSION OF MAGE GENES BY NON-SMALL-CELL LUNG CARCINOMAS", PAGES 826-829, SEE ENTIRE DOCUMENT.	1-10
Y	WO, A, 92/20356 (BOON ET AL) 26 NOVEMBER 1992, SEE ENTIRE DOCUMENT.	1-10
A,P	US, A, 5,342,774 (BOON ET AL), 30 AUGUST 1994, SEE ENTIRE DOCUMENT.	1-10

Further documents are listed in the continuation of Box C.

See patent family annex.

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"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

01 MAY 1995

Date of mailing of the international search report

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Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231  
Facsimile No. (703) 305-3230

Authorized officer *Editha F. Lee, Jr.*  
EGGERTON CAMPBELL  
Telephone No. (703) 308-0196